EDITOR'S VIEWPOINT

Cowan (p. 824), on the basis of a statistical analysis of patients' reaction after use of Xylocaine anesthetic, concludes that most dentists use excessive dosages of local anesthetics. He has prepared a suggested table of dosages for various procedures and hints that manufacturers should provide smaller carpules. This example of clinical research should encourage others.

Sallay (p. 840) investigated the periodontal condition of 155 adults with cyanosis due to acquired cardiac disease and found a correlation with alveolar atrophy starting in the region of the mandibular first incisors. In a second group, 33 individuals of 3 to 28 years with congenital cardiac defects, alveolar atrophy was observed in 24 of 25 exhibiting cyanosis. The condition resembled periodontosis. While some have questioned the existence of periodontosis as an entity, this study indicates a relationship between periodontal disease and cyanosis, and Wiener, Karshan, and Tenenbaum (p. 875) suggest its relationship with hormonal disbalance. These may be among the numerous systemic factors which reduce the reparative and maintenance ability of the periodontium. Some prefer to consider this as a factor in production of disease, others consider it a disease entity.

The potential value of a filling material capable of bonding to tooth surfaces is unquestionable. While no such materials are yet available for use in the mouth, the search for them in institutional and commercial laboratories holds promise. Buonocore, Wileman, and Brudevold (p. 846) found that acid etching of dentin surfaces before application doubled the adhesion of materials (possibly by increased mechanical adaptation) and have developed a resin which, in vitro, offers possibilities of "bonding." As the studies continue in many laboratories, we can hope for useful, bonding types of restorative materials.

Mucoceles usually are described as retention cysts lined by epithelium and caused by ductal obstruction. Bhaskar, Bolden, and Weinmann (p. 852) first attempted to produce mucoceles by ligating the salivary gland ducts of mice. Even after 9½ months no mucoceles were produced but glandular changes, other than retention cysts, were observed. In a companion study Bhaskar, Bolden, and Weinmann (p. 863) severed the salivary ducts in 100 animals (mice and rats) and produced mucoceles in 8 instances. The mucoceles were not lined by epithelium, as was also true for most mucoceles they examined from human subjects. The mucoceles were of 2 types, large cystic cavities and numerous microcysts. This contribution to the pathology of mucoceles offers some new concepts and emphasizes the value of the correlated experimental and morphologic approach to pathology.

Weiner, Karshan, and Tenenbaum (p. 875) investigated the ovarian function (as judged by vaginal smears and temperature) of 33 females with periodontosis. The severity of the periodontosis varied from slight to severe. Of the 19 females with "good" ovarian function, 8 had slight, 7 moderate, and 4 severe periodontosis. At the other end of the scale, of the 6 females with poor cycles (1 only 13 years old), and inovulatory, 1 had slight, 1 moderate, and 3 severe periodontosis. There appears to be some correlation but certainly not any definite relationship. At the same time, oral and vaginal smears were studied simultaneously in 16 females, of whom 11 showed good correspondence and 5 none. As one might expect, the ovarian hormonal cycle may be reflected, to some degree, by changes in the oral epithelium, but the relationship often is indefinite.

Meredith and Hopp (p. 879) utilized the opportunity for a longitudinal dental study in 77 children enrolled in a long-term research program. The dental arch widths were measured on casts made at 4, 6, and 8 years of age. They confirmed the impression that the maxillary arch is wider than

the mandibular (3.1 mm.), found that the male arch is wider than the female arch (1.9 mm.), and that the arch is wider (1.7 mm.) at 8 than at 4 years. The maxillary arch is slightly more variable than the mandibular. There was considerable individual variation.

A correlation between the cuspal and palatal inclines of the teeth and the inclined plane of the glenoid fossa has been postulated and used in descriptions of functional relationship. Koyoumdjisky (p. 890) devised a measuring instrument for these planes and utilized it on 10 skulls. Her findings, while not conclusive, show much more suggestion of a relationship between the palatal inclined planes of the upper anterior teeth and the glenoid fossa plane than that of the occlusal planes of posterior teeth and the fossa's slope. The results indicate a definite need for a more expanded study in order to support or reject the theory.

Goose (p. 902) measured the first maxillary incisor and devised an index to indicate its variation. It is suggested that measurements on this tooth may be used to study genetic factors in relationship to the dentition.

Sainsbury (p. 909) examined the teeth of 5-year-old children for caries and for structural defects and plotted these findings against the temperature at the time of their births, a year of great seasonal variation. Some significant correlation is suggested for deciduous teeth. This type of study may well be extended to isolated groups in other regions and caries, structure, and weather at time of gestation evaluated for both permanent and deciduous teeth.

Ramnarine and Altinsel (p. 914), in a brief screening study, found indications that oxytetracycline (Terramycin) applied at the concentration of 250 mg. per cubic centimeter of solvent may be effective in sterilizing carious dentin. The study should be supported by clinical investigations.

The nerve supply to teeth appears to be a subject that should have been firmly established many years ago. However, much of the published material was descriptive on the basis of a few specimens rather than on the results of investigations on a reasonably large number of skulls. McDaniel (p. 916) studied the nerve distribution to the maxillary teeth in 50 decalcified skulls. The posterior superior nerve had 1 to 3 branches distributed in a 45:30:25 per cent ratio. Only 15 per cent of the specimens showed the so-called classical description for the middle superior dental nevre and, while the anterior superior dental nerve most frequently was "classical" in distribution, 36 per cent of the skulls showed branches to the premolar teeth. Almost half of the specimens had superior dental plexuses. The findings explain some reactions to local anesthetics and some problems in diagnosis of pain in the premolar region.

Shafer and Muhler (p. 922) have demonstrated a relationship between thyroid gland function, as controlled experimentally, and salivary gland histology. A previous study of theirs had shown that thyroid administration reduced rat caries and that thiouracil increased dental caries in the rat. These experiments strongly suggest that the thyroid influences dental caries in the rat through salivary gland influences. It is interesting that fluoride administration, often suggested as having an influence on thyroid function, did not affect the salivary glands in these studies.

Irving (p. 930), from study of the reaction of the enamel organs of the continuously erupting incisors of rats fed high levels of cod liver oil or hake liver oil with and without L tocopherol, and from comparisons with results of reactions of the enamel organ in animals under influence of other toxic substances, has concluded that the enamel organ of continuously erupting teeth varies from point to point in its sensitivity to different toxins and in its nutritional requirements. Translated into terms of human teeth this might indicate that any given toxic substance or nutrient may affect enamel formation differently if given at different times, or that different substances may affect it differently at the same time.

The antibacterial action of fluorides has been reported by several authors. Shiota (p. 939) tested various concentrations (50 to 500 ppm) of sodium fluoride against 20 homofermentative strains and 20 heterofermentative strains of lactobacilli. There was a variation in the resistance of the different strains to fluorides but, in general, they fall into 3 groups. As the pH of the media was lowered, the effectiveness of the sodium fluoride was reduced. The possible

selectivity of sodium fluoride may account for failure to reduce salivary lactobacillus counts with fluorides, although caries may be inhibited.

The Proceedings of the British Division of the International Association for Dental Research are included on page 947. The qualitative, as well as the quantitative, improvement is most commendable. The increasing dental research around the world re-emphasizes the ever present need for a large outlet for dental research publications.

The expanding activities of this group deserve our praise and congratulations. The variety of interests and thorough investigations are indicative of the expanding efforts in dental research.

H. B. G. R.

A CLINICAL EVALUATION OF XYLOCAINE ANESTHESIA

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XYLOCAINE (ω-Diethylamino-2,6-dimethylacetanilide) resulted from the experiments of Erdtmann and Lofgren. Their work paved the way for Lofgren and Lundquist to produce the anesthetic in 1943, and the first dental reports based on 5,000 injections were published by Bremer, Ekmanner, Persson and Strondberg.[1] It was claimed that Xylocaine is superior to procaine and Monocaine in anesthesia, penetration of the jaw was very rapid, and side reactions minimal. Gruber[9] compared Xylocaine with procaine and found the former more selective for sensory nerves, thus reducing motor involvement, while Hanson and Hingson[11] used Xylocaine intravenously in large amounts for labor and delivery, and abdominoperineal surgery without complications.

Björn and Huldt,[2] in 1947, devised an electrical method for measuring efficiency of an anesthetic agent. Stimulating the incisal tip of a tooth with an electrode, they measured the pain threshold before and after injection by varying a rheostat. They compared Xylocaine and procaine in concentrations of 0.5 to 4.0 per cent for a standard dose, and Huldt,[12] in 1953, extended the study further, examining concentrations rather than dosage.

Thexton, Wishart, and Baxendine,[19] in 1950, analyzed 250 Xylocaine injections and suggested a maximum dose of 2 ml. (34 minims) for jacket crowns and surgical work, and a minimum of 15 minims for submucous infiltrations for such work as pulp extirpation. Ofstad[15] pointed out that calculation of dosage of medicaments, and of their toxic doses, is related to body weight. Data derived from animal experiments corrected by clinical observation furnish a very insecure basis for estimating a safe dose for human patients.

It is highly probable that, largely due to a desire to assure rapid and effective anesthesia, the average dosage of anesthetic for dental treatment, especially in simple infiltrations, is greater than necessary, and that there is need for a guide to dosage in everyday procedures.

This investigation was undertaken: (1) to establish from a clinical standpoint, whether or not Xylocaine is superior to procaine as a local anesthetic; (2) to assess accurately the onset times for each anesthetic agent in various regions of the mouth; (3) to investigate whether any relationship exists between dosage and site on one hand, and duration and extent on the other; and (4) to demonstrate from the data collected, dosage-time regression lines for each agent, and to establish statistical confidence limits for time, given various dosages.

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METHODS AND MATERIALS

A group of 300 patients was examined, cases being taken at random, except where the possibility of identical work on each side of the mouth for control purposes presented itself.

A total of 329 injections was administered, 160 procaine, 169 Xylocaine, the treatment being routine work in all regions of the mouth. No patient received any information whatever about the nature of the anesthetic being used, and was merely asked if he would cooperate in a timing survey. All injections were made by myself.

Agents.—Two solutions were used throughout: (1) Xylotox (2 per cent with Adrenalin $\frac{1}{80,000}$ (a proprietary brand of ω-diethylamino-2,6-dimethyl-acetanilide manufactured in Great Britain), and (2) procaine (2 per cent with Adrenalin $\frac{1}{40,000}$. Information was recorded on special forms with columns for the various data contained in later tables.

Assessment.—Onset times were measured from the moment of insertion of the needle until the appropriate manifestation of anesthesia for that region, thus taking into consideration injection time (10 to 30 seconds).

Actual onset was noted in stages: (1) infiltrations: (a) gingival anesthesia over tooth involved, and (b) operative anesthesia; (2) mental block: (a) lip anesthesia, and (b) operative anesthesia; (3) mandibular block: (a) tongue anesthesia, (b) lip anesthesia, and (c) operative anesthesia. Where lip or tongue was involved, the first symptom of tingling or numbness was recorded as onset. Operative anesthesia meant: (1) elimination of all sensitivity from a cavity which had been painful to probing or drilling. Probing was carried out first where possible, and as soon as it ceased to hurt, drilling was commenced. If further sensitivity was encountered, the time was measured up to the point where that was eliminated. (2) Extractions were measured to painless application of forceps and rocking of the tooth, and surgical procedures to elevation of periosteum.

Anatomical extent was determined by stroking the gingiva with a sharp probe after operative onset time was established. For obvious reasons the test was only for gingival anesthesia, and it has been expressed in units similar to those used by Angle in his orthodontic classification. A unit is the average mesiodistal diameter of a premolar [7 mm. (Sicher)] and the measurement was applied whether or not all teeth were present. One molar = 2 units.

Duration was measured to the nearest 15 minutes on the patient's observation.

Side effects such as pallor, sweating, dizziness, thready pulse, or actual fainting were recorded if they occurred, and a watch was also kept for later reactions such as pain, at the anesthetic site, headache, and prolonged anesthesia.

The vast majority of treatments were fillings, since they offered best means of comparison and timing, but other treatments included pulp extirpation, jacket crown, and inlay preparations, extractions (simple and surgical), apicectomy, gingivectomy, and surgical treatment of cysts.

The degree of anesthesia was based on the patient's reaction to probe or drill. The following classification was used:

+1 gingiva only,

+2 slight reduction of pain for drilling, or application of forceps,

+3 occasional twinges during drilling. Pain on rocking tooth in extraction, and

+4 complete anesthesia.

All Xylocaine cases gave +4 anesthesia, but as all procaine did not, a time limit to allow a +3 to become +4 was adopted. It was called "maximum improvement time" and is noted on the various procaine tables. It is based upon a reasonably practical clinical working time in each group.

Group I. Infiltrations: using 0.25, 0.5, 0.75, 1.0, and 1.5 ml.

Group II. Mental blocks: using 0.25, 0.5, 0.75, 1.0, 1.5, and 1.75 ml.

Group III. Mandibular blocks: using 2.0 ml.

Group IV. Patients with work carried out on one side of the jaw with procaine, and on the same, or the other side, closely similar work with Xylocaine. Except for the 0.25 ml., the same number of injections were recorded for each agent.

Statistical comparisons were made, in the first place, directly for each type of injection given, and secondly, the over-all findings were re-examined under the headings common to all the group.

Group I is the largest group (87 Xylocaine, 68 procaine) and covers considerable dosage range for analysis. Unfortunately exact details of each injection and the work done have had to be omitted for space reasons, but similar details are shown in the other tables.

STATISTICAL REPORT ON FIGURES OBTAINED CLINICALLY

Group I. Infiltrations.—Statistical methods were applied to investigate from the onset and operative time figures, and the dosages: (1) the relation between dosage and time (T) from insertion of needle to operative anesthesia, (2) the dispersal of these T's around the mean value, separately for each drug, (3) the mean values of the T's for both drugs and whether it can be claimed that one drug becomes operative sooner than the other, and (4) confidence limits.

Average onset times (operative): The averages for Xylocaine suggest that the onset times for dosages less than 0.75 ml. are higher than for dosages of 0.75 ml. or more. The Student t-test was applied to check the significance of the figures, and the probability that the difference in means of 16″ or more could have arisen through sampling errors was approximately 10 per cent. It cannot therefore be stated conclusively that the average onset time for low dosage is higher than for greater dosages, but when it is remembered that onset time for dosage less than 0.25 ml. tends to infinity it is reasonable to infer

TABLE I
AVERAGE OPERATIVE ONSET TIMES IN MINUTES AND SECONDS

DOSAGE IN ML.	XYLOCAINE	PROCAINE	NO. OF CASES X.	NO. OF CASES P.
0.25	1'34" } Average	—	20	—
0.50	1'23" } 1'29"	3'19"	21	21
0.75	1'10" } Average	3'58"	6	6
1.0	1'14" } 1'13"	3'14"	36	36
1.5	1'15" }	2'48"	4	5
Totals			87	68
	Grand average (Xylocaine)	1'21" (operative)		
		17" (gingival)		
	Grand average (procaine)	3'17" (operative)		
		44" (gingival)		

as follows: Onset time decreases as dosage increases, up to 0.75 ml., and thereafter remains constant. Further, the increase in onset time for lower dosages is not significant as compared with the effect of the drug.

The procaine results were also analyzed (Analysis of Variation technic) but no significant evidence of dosage-onset time was established.

Dispersal of T's around mean value: The data allows estimates of the variances (variance = square of standard deviation) of the times of procaine and Xylocaine to be made for dosages of 0.5 ml. and upward. These were found to be 2291" and 6134", respectively and were tested, using the Snedecor F. Distribution Tables, to find whether they indicate that Xylocaine variance is "significantly" lower than procaine variance. On entering the tables with the ratio of the 2 variances it can be said with 98 per cent confidence that the true procaine variance is at least 1½ times greater than that for Xylocaine.

Mean values of T's: Xylocaine mean T was 1' 21", and procaine counterpart was 3' 17". A modified Student t-test showed that the probability of sampling errors was 1 chance in 1,000. Similarly Xylocaine mean T [1' 29" for an average dosage of 0.38 ml. (0.25 to 5.0)] is significantly less than procaine mean T, based on an average dosage of 0.86 ml.

TABLE II

DRUG	AVERAGE T	LIMITS FOR AVERAGE T (98% CONFIDENCE)	APPROXIMATE UPPER LIMITS OF T
Xylocaine	1'21"	1'8" -1'34"	3'25"
Procaine	3'17"	2'50"-3'45"	7'10"

Using the results from the 3 analyses, confidence belts were estimated for each drug for average T's, and the approximate figures for the upper limits of T. The figures based upon average dosage of 0.86 ml. are shown in Table II.

TABLE III

DRUG	DOSAGE (ML.)	MEAN ONSET TIME	LIMITS FOR MEAN ONSET TIME (98% CONFIDENCE)	EXTREME ONSET TIME
Xylocaine	0.25-0.5	1'29"	1'8" -1'50"	3'40"
	0.75-1.5	1'13"	57"-1'29"	3'5"
Procaine	0.5 -0.75	3'27"	2'47"-4'6"	6'50"
	1.0 -1.5	3'11"	2'32"-3'50"	6'15"

A breakdown to show the figures for dosages of less than 0.75 ml. and greater than 0.75 ml. is shown in Table III.

Xylocaine acts more quickly than procaine (on average 2 minutes sooner) and its variations from average are considerably less. Fig. 1 shows regression lines for the 2 drugs for time (ordinate) and dosage (abscissa).

Group II. Mental Blocks.—Group II (Xylocaine 29, procaine 30), like Group III, is confined to a specific area which thus permits direct comparisons to be made. Detailed data are again omitted for space reasons, but the condensed data are given in Table IV.

TABLE IV
OPERATIVE TIMES

DOSAGE	XYLOCAINE	PROCAINE	CASES	
			X.	P.
0.5 ml.	1'34"	3'44"	8	12
1.0 ml.	1'47"	3'16"	12	12
Grand Average	1'42" (operative) 39" (lip)	3'30" (operative) 48" (lip)		

By methods similar to those applied for Group I, statistical confidence limits were established (Table V).

TABLE V

DRUG	AVERAGE T	LIMITS FOR AVERAGE T (98% CONFIDENCE)	APPROXIMATE UPPER LIMIT OF T
Xylocaine	1'40"	1'12"-2'12"	4'0"
Procaine	3'30"	2'40"-4'10"	7'30"

The same relationship between the two drugs is maintained for the slightly longer onset times obtaining in this injection. Xylocaine acts more rapidly, its variations around average times are less, and its dosage is relatively unimportant.

Group III. Mandibular Blocks.—Mandibular blocks (Xylocaine 50, procaine 50) are regarded as the most important because experience shows that this injection produces more anesthetic variations in practice than any other. A sharp contrast is apparent in the respective findings, and although it is not possible to include details, application of the same test as for the data in Group I produced the figures in Table VI.

TABLE VI
MANDIBULAR BLOCKS

DRUG	AVERAGE ONSET TIME			LIMITS FOR AVERAGE OPERATIVE TIME (98% CONFIDENCE)	APPROXIMATE UPPER LIMITS FOR OPERATIVE T
	TONGUE	LIP	OPERATIVE		
Xylocaine	1'36"	2'14"	4'30"	3'50"-5'10"	8'30"
Procaine	2'36"	2'58"	7'18"	6'18"-8'18"	14'27"

Various combinations of lip and tongue times were collated in order to discover whether there was any relationship between them and operative time, or whether any indication as to when operative time would ensue could be derived in advance clinically from lip and/or tongue times, but no such relationship could be established.

DISCUSSION

Onset Times.—It is opportune to explain why so much stress has been placed on the exact measurement of onset times from the time of needle insertion. It was obvious at an early stage that Xylocaine was so rapid acting that in cases when operative time is effective in less than 1 minute, injection time must be considered since, as it will vary directly with the amount used, it cannot be disregarded as a constant which will not affect the final figures.

Thus, while the slower procaine times would absorb injection times, it would be impossible to draw up a comparative graph for Xylocaine which would show time-dosage relationships without this information. Another result will be that onset figures will differ from those set out in other reports. Björn and Huldt[2] describe "analgesia latency" which they define as "the

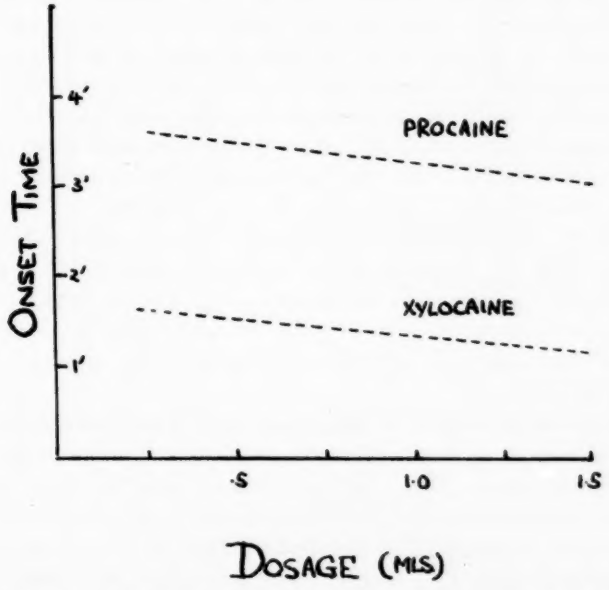

Fig. 1.—Regression lines for procaine and Xylocaine for time (ordinate) and dosage (abscissa).

time in minutes between the end of the injection and the appearance of complete analgesia." They point out themselves that it is only in the nature of a rough estimate, since if it ended in 50 seconds, the latency was recorded as "0," while 65 seconds gave a reading of "2." Hence their deduction that analgesia latency is not statistically different between Xylocaine and procaine is, in these circumstances, hardly feasible. Likewise, Thexton, Wishart, and Baxendine[19] give only round figures for onset times (maxillary infiltration: 2 to 3 minutes, mandibular and mental block, 3 to 5 minutes average). Dubin and Foner,[6] in 1952, in a short survey of 3,000 injections of Xylocaine (2 per cent with Adrenaline 1/100,000) found satisfactory anesthesia in every case compared with a failure rate of 20 per cent with procaine (Table VII).

TABLE VII
COMPARISON OF XYLOCAINE AND PROCAINE (DUBIN AND FONER)

DRUG	INDUCTION TIME INFILTRATION	BLOCK	DURATION (HOURS)
Procaine	3-5 minutes	5-10 minutes	1-1½
Xylocaine	1'50"	2'20"	2-4

The block induction times appear a little optimistic for mandibulars, and no mention is made of injection time, but it is noteworthy that they record Xylocaine times in minutes and seconds, and procaine in a much wider range.

In the present series infiltrations covered the widest dosage range and, with the mental blocks, provided the material for the time-dosage graph (Fig. 1.) The primary conclusion from these figures indicates clearly that doses of 1.5 to 2.0 ml. are rarely, if ever, required for Xylocaine infiltrations. For average treatment (fillings, extractions) the dose appears to be in the region of 0.75 ml., but lower dosages still are adequate for 2 adjacent teeth. The upper third molar is usually a simple tooth to extract, and in 8 cases an attempt was made to find the fastest time from insertion of needle to completion of extraction which could be accomplished with 0.5 to 1.0 ml. of Xylocaine. It was found possible to complete the total operation in a time varying from 90 to 50 seconds. The 0.25 ml. infiltrations with Xylocaine were also interesting since the onset times compare favorably with procaine dosages of 1.5 ml. An attempt to use similar dosages of procaine was a complete failure.

Xylocaine, 0.25 ml., appears to be the ideal amount for a single-rooted tooth but it is also effective in bilateral mental blocks (0.25 each side) for gingivectomy, because much less demand on anesthesia is involved in that treatment. In such cases the shorter duration and the lessened motor effect are most gratifying.

Yet another useful result of this dose is the identification of the causative tooth in neuralgic pain involving 2 or more adjoining teeth with large restorations in the upper jaw. An injection of less than 0.25 ml. over the apex of a single, or between the buccal apices of a multirooted tooth will produce anesthesia, and stoppage of the pain within the confidence limits, if it derives from that tooth (1'8" to 1'50"). Since extent is limited at this low dosage (4 units) the onset time would be outside the confidence limits for a neighboring tooth.

Mental blocks (Table IV) also presented comparable onset times as the injection is easily carried out with accuracy and the position standardized. The ratio of Xylocaine to procaine times through a considerable series of dosages remains statistically significant at 2:1.

Mandibular block failures have been ascribed to errors in technic, ramus contour, aberrant nerves,[4] and supplementary innervation, quite apart from inadequate anesthetic solutions.

Jeffries[13] stated that the lip is the guide to anesthesia, and its anesthesia is followed by the "easy areas" of lower 4 to 8 in that order. The most difficult part to anesthetize is the mesiobuccal quarter of the molar, the remaining three-quarters constituting the "easy areas." Seldin (quoted by

Jeffries[13]) explained that the fibers traveling the greatest distance are in the central core of a nerve, and if the outer fibers receive all the anesthetic in a block, the fibers traveling to the pulp may not be affected, resulting in sensitivity. Using procaine, Jeffries established that the lip was usually numb in 4 minutes, and where molar anesthesia was incomplete, a repeat injection of 1.0 ml. would saturate the innermost fibers.

In a number of the procaine blocks in the present series, +3 degree referred to this "difficult area," but this did not occur with Xylocaine. The indirect technic was used in all cases, and it seems likely that with Xylocaine a fair amount of latitude in positioning the needle is permissible without loss of efficiency, because even if positioning appeared less than perfect, anesthesia always developed.

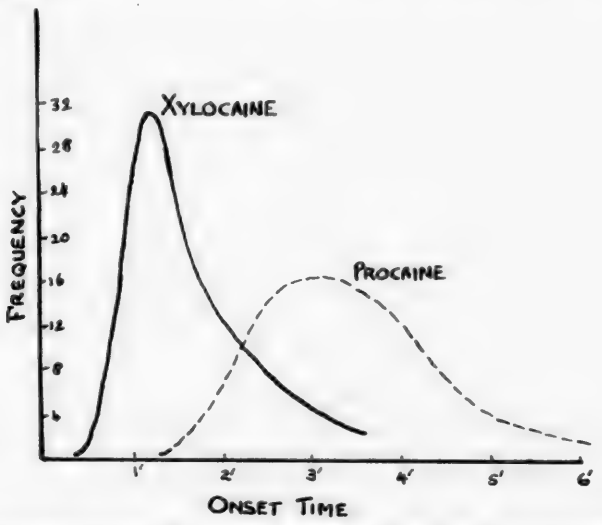

Fig. 2.—Frequency distribution of operative onset times for local infiltration of procaine and Xylocaine.

Many patients receiving Xylocaine for the first time volunteered the information that onset and depth of anesthesia were more rapid and profound than with previous injections (usually procaine or Monocaine) while others who had been receiving Xylocaine regularly invariably expressed surprise at the slow onset when they were used in the control group with procaine. Greatest advantage of this rapid onset is that it brings block time into the time range of infiltration. Xylocaine: procaine ratio in this group was 1.6:1.

Fig. 1 shows almost parallel lines for the 2 drugs, and one may assume a rise in the time as dosage lowers and time increases, more marked in the procaine line. The onset figures for the failed procaine anesthetics were inserted as the time at which the final recorded degree first developed, although a much longer delay was involved clinically to see if any late improvement might develop. If it were possible to incorporate this information in Fig. 1, the disparity between the 2 lines would be clearer.

Fig. 2 shows the frequency distribution of operative onset times for local infiltrations for both drugs. It is extracted from the figures for all the dosages involved, and demonstrates that for the mean time of 1′ 22″ (Xylocaine), variation is very slight, and onset time highly predictable. For procaine a much flatter curve develops for the mean time (3′ 15″) and the extreme times are not only longer (minimum time corresponds to the Xylocaine mean) but cover a much wider range (5 minutes, against 3 minutes for Xylocaine). Fig. 2 is based upon an equal number of cases for both drugs and direct comparison is permissible. The important fact emerges that time variation against dosage is extremely slight for Xylocaine, but there appears to be an improvement up to 0.75 ml., after which higher dosages for infiltration do not affect onset times. Comparison of over-all onset times is expressed by the ratio procaine to Xylocaine of 2.5:1.

Extent.—The extent of anesthesia was only measured for gingival anesthesia, but where additional fillings were required in neighboring teeth under infiltration, the extent with Xylocaine was always greater.

Huldt and Björn[2] give the following figures where injections were made over the second incisor:

	First Incisor	*Second Incisor*	*Canine*
2 per cent Xylocaine	100 %	100 %	75.6%
2 per cent procaine	78.5%	56.7%	13.5%

Xylocaine: procaine ratio for all three teeth together, is 5:1. Extent in this region of course is limited by the cross fibers at the midline, and more information might be obtained in other areas. An analysis of the present series is shown in Table VIII.

TABLE VIII
EXTENT OF ANESTHESIA (MAXILLA) IN UNITS

SITE	2% PROCAINE		2% XYLOCAINE	
	1.0 ML.	0.5 ML.	1.0 ML.	0.5 ML.
1. Needle inserted over *8*	4.75	3.5	8	4
2. Needle inserted over *7*	4.5	3	6	6
3. Needle inserted over *6*	5.8	4	6	6
4. Needle inserted between *67* or *78*	4.6	—	7	—
5. Needle inserted over *4* or *5*	3	3	4.3	4
6. Needle inserted over *3*	5	3	5	4
7. Needle inserted over *2*	3	3	3	3

Except when limited by the fauces (*78*) or midline (*12*), spread may be taken as being equidistant mesially and distally from the injection point. Maximum figures occur for the molar region because the looser tissues allow greater diffusion and also because of the numerous bony foramina which promote absorption.

Figures for the mental blocks: procaine $\frac{138}{32} = 4.31$ units, and

Xylocaine $\frac{175}{32} = 5.46$ units.

A difference of approximately the width of one premolar was observed.

Figures for the mandibular blocks: procaine $\frac{464}{50} = 9.28$ units, and

Xylocaine $\frac{497}{50} = 9.94$ units.

A difference of about one-half a premolar was observed.

Xylocaine is hardly affected by a drop from 1.0 to 0.5 ml., but the change with procaine is marked. In both cases the greatest drop occurs in *8* and *3* areas (Table VIII). Fig. 3 shows regression lines for dosage/extent for both anesthetics from the over-all statistical analysis, and the effects are summarized in Table IX.

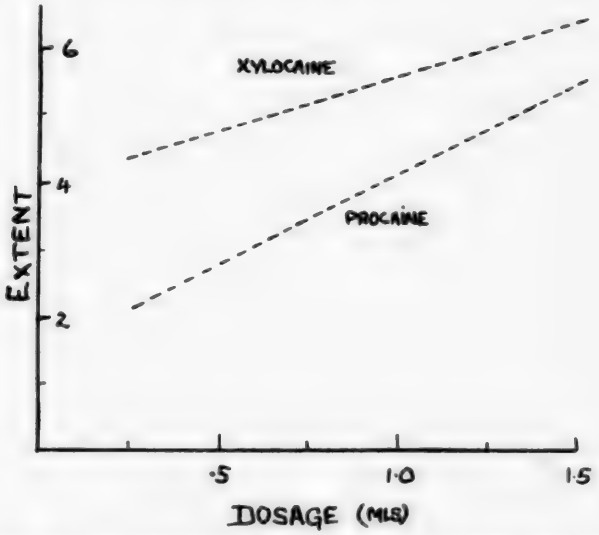

Fig. 3.—Regression lines for dosage/extent for procaine and Xylocaine from over-all statistical analysis.

Variation of extent with dosage was established by Analysis of Variance, and the longer extent (for equal dosage) of Xylocaine was verified by the Student t-test. A considerably lower quantity of Xylocaine than procaine will produce an equal extent.

Degree of Anesthesia.—Even for single teeth the degree of anesthesia for 0.25 ml. infiltrations of procaine was so poor that the controls had to be abandoned. Since no Xylocaine injection produced a degree of less than +4, an attempt was made to relate those Xylocaine cases which had shown a slower onset time than the statistical averages, to procaine injections showing less than +4 degree. There was, however, no evident connection between work done, or tooth treated, or surface treated for any area or dosage, and inadequate procaine depth of anesthesia.

Procaine failures are in the region of 30 per cent in this series, a higher rate than that quoted by Tainter[18] (20 per cent), who used a similar solution, or Björn and Huldt,[2] and Dubin and Fraser,[6] who confirm his

figure. It is partly explained by the fact that even when the work could be done in +3 cases, with only a small amount of pain, it was recorded as a failure. Allied to this is Huldt's observation that procaine is usually regarded as being satisfactory in everyday use because much larger doses of procaine are generally used in practice. Doses of the order of 0.25 to 0.75 ml. as used in this series do not appear in other reports where procaine was employed.

TABLE IX
EXTENT OF ANESTHESIA VS. DOSAGE

DOSAGE (ML.)	DRUG	
	XYLOCAINE UNITS	PROCAINE UNITS
1.5	7.2	5.0
1.0	5.6	4.3
0.5	5.3	3.0
0.25	4.0	—

Xylocaine produces a higher degree of anesthesia in all dosages than the procaine equivalents, and the 0.25 ml. injection is equivalent to 4 to 6 times that dose of procaine (Table XII). From the detailed figures of infiltrations, the most readily anesthetized tooth appears to be the upper premolar.

Duration.—It is more important that duration should outlast operative procedure than that the slight physical discomfort of extended anesthesia for an hour or 2 longer should be eliminated, and both anesthetics tend toward that goal.

The local infiltration cases were examined statistically to test the effect of drug and dosage on duration, and the results are summarized in Table X.

TABLE X
EFFECT OF DOSAGE ON DURATION

DOSAGE (ML.)	DURATION	
	XYLOCAINE	PROCAINE
1.5	3 hrs. 6 min.	2 hrs. 15 min.
1.0	2 hrs. 45 min.	2 hrs. 15 min.
0.75	2 hrs. 20 min.	1 hr. 46 min.
0.5	2 hrs. 28 min.	1 hr. 42 min.
0.25	1 hr. 43 min.	—

The slight discrepancy in the 0.5 ml. dose in Xylocaine may be accounted for by the fact that there was a larger number of these cases, and their variation was slightly greater. In any event, it is evident with both agents that duration decreases as dosage decreases. It is noted that Xylocaine decreases very slightly down to 0.5 ml., but considerably with 0.25 ml.

Mandibular block times (procaine 3 hours, Xylocaine 3½ hours) compare with the figures of Björn and Huldt, whose timing was more accurately carried out on dental students. A feature of its end point is that, with Xylocaine, numerous patients commented on its sudden disappearance. After slight reduction in intensity it goes, "One minute it's there, the next it's gone."

Motor Nerve Involvement.—Drooping of either lip is seen in anterior injections due to loss of motor control, and it is a troublesome side effect which, in spite of warnings, still leads to biting of the lip. When a low dosage of Xylocaine is employed, however (0.5 to 0.25 ml.) the reduction in lip involvement is very noticeable, and this can be best estimated by observing women patients reapplying lipstick after completion of treatment.

The explanation of the reduced motor effect lies largely in the reduced amount of anesthetic available for the motor nerve with these small doses, but Gruber,[9] who evaluated the motor involvement with mental blocks, believed that Xylocaine shows, in addition, greater selectivity for sensory nerves.

Support for this view is forthcoming in the present series where it explains the high degree of success in mandibular blocks even where technic left something to be desired for one reason or another. For example one case showed normal mandibular block time although the nerves had been grossly displaced by a large dental cyst. In addition a block of Meckel's ganglion was inadvertently produced while giving a simple tuberosity infiltration with 1.0 ml., although the needle was only inserted about 12 mm. and must have been a considerable distance from the ganglion.

Pulse Rates.—Pulse rates were noted before and after every anesthetic that was given, but the variation, such as it was, was most irregular. So many other factors, mainly psychic, operate in this sphere that one cannot draw any conclusions of value.

Toxic Effects.—There were few toxic effects with either drug, but when they did occur they were distressing for the patient, and time consuming and often worrying for the operator.

With Xylocaine, nausea occurred in 2 cases (one a child), pain at the anesthetic site in 3 cases, and prolonged anesthesia in 2 mental blocks where, almost certainly, injection had been directly into the nerve. Two patients with history of anesthetic sensitivity were injected with Xylocaine with no ill effects whatsoever.

Gruber,[9] commenting on the comparative toxicity of Xylocaine, quoted the work of Goldberg, who calculated its absolute toxicity as 1.5 to 4.0 Gm. subcutaneously. He also showed that up to 8 per cent Xylocaine had no irritant effect on the rabbit cornea.

Gordh[7] described 2 cases of overdosage in human surgical patients, both of whom responded to treatment. The doses were of the order of 150 ml. of 2 per cent Xylocaine (for paravertebral block and thoracoplasty, respectively). In each case, stupor with clonic spasms of the face, extremities, and respiratory muscles set in. Intravenous narcosis with 5 per cent Narkotal plus oxygen inhalation produced immediate relief.

Since this series was prepared, the writer has seen 1 severe case of Xylocaine toxicity. A patient was referred for completion of a failed extraction of $\overline{7}$, attempted the day before. He was in considerable pain and a nerve block of 2 ml. of Xylocaine (with $\frac{1}{80,000}$ Adrenalin) was given. Anesthesia was normal in onset, and profound, but after a flap had been reflected and

bone removal commenced the patient's color was seen to change, and he became ash-gray and pulseless, with very shallow respirations. Almost immediately after this, convulsive spasms began in the arms and legs, and he became momentarily unconscious. Simple measures for shock were introduced, and then he was removed to hospital where the operation was later completed under general anesthetic. The case is reported only to describe the convulsive attack combined with shock which would make the administration of stimulants dangerous.

With the foregoing information established, a short series of cases constituting Group IV was examined, wherein direct comparison was made in the same mouths.

TABLE XI
DIRECT COMPARISON ON SAME OR OPPOSITE SIDES OF A PATIENT OF PROCAINE 2% AND XYLOCAINE 2%

CASE NO.	ANESTHETIC	AMOUNT (ML.)	WORK	ONSET			DURATION (HRS.)	DEGREE
				GUM	OPERATIVE			
394	Procaine	1.0	$\overline{4^{o}4^{d}}$ |	1′	3′30″		1½	+4
	Xylocaine	1.0	| $\overline{4^{o}4^{d}}$	30″	1′		2	+4
398	Procaine	1.0	| $7^{o}m7^{o}d8^{o}$	1′	7′		1¾	+3
	Xylocaine	1.0	$\overline{8^{o}7^{o}m7^{o}d}$ $\overline{6^{m}6^{od}}$ |	30″	3′		2	+4
447	Procaine	0.5	$\overline{3}$ | ^{m}	30″	5′30″		2	+3
	Xylocaine	0.5	| $\overline{2^{d}}$	10″	2′30″		2	+4
				Lip	*Operative*			
43	Procaine	1.0	| $\overline{4^{c}5^{d}}$	1′50″	2′		1	+3
	Xylocaine	1.0	$\overline{4^{c}5^{c}}$ |	30″	45″		2	+4
305	Procaine	0.5	| $\overline{4^{o}}$	25″	4′30″		2	+2
	Xylocaine	0.5	$\overline{4}$ | ^{o}	10″	1′		1½	+4
				Tongue	*Lip*	*Operative*		
93	Procaine	2.0	| $\overline{8^{o}7^{b}}$	3′	3′20″	5′30″	3	+4
	Xylocaine	2.0	$\overline{6^{o}4^{c}}$ |	1′20″	2′20″	3′30″	4	+4
335	Procaine	2.0	| $\overline{7^{m}7^{o}8^{o}}$	2′55″	3′20″	9′50″	2	+4
(same side)	Xylocaine	2.0	| $\overline{5^{o}5^{d}6^{o}}$	1′	2′	3′	3	+4
358	Procaine	2.0	$\overline{7^{o}8^{o}}$ |	1′50″	2′30″	5′50″	3½	+4
	Xylocaine	2.0	| $\overline{7^{o}8^{o}}$	1′30″	2′30″	3′15″	3	+4

Huldt showed an efficiency rate of 97 per cent with $\frac{1}{40,000}$ Adrenalin, 90 per cent with $\frac{1}{100,000}$, and 65 per cent with $\frac{1}{250,000}$, so the effect of this drug upon the anesthetic must not be ignored. If a dose of 0.5 ml. Xylocaine with Adrenalin $\frac{1}{80,000}$ is equivalent to a dose of 1.5 ml. procaine with Adrenalin $\frac{1}{40,000}$, not only is the volume of the injection reduced to $\frac{1}{3}$, but the actual amount of Adrenalin is only $\frac{1}{6}$ that of the procaine dose.

While this is not of medical importance in the vast majority of cases, and is regarded as a "calculated risk," the odd case where a heart condition has not been diagnosed, or where hypersensitivity exists, may occur, and the

danger may suddenly be magnified. Every year one reads of the rare case of collapse, or even death from such a cause, and, as Gordonoff[8] has noted: "Most of the general reactions observed in the use of procaine-Adrenalin solutions are undoubtedly due to the Adrenalin, and many unpleasant reactions could assuredly have been avoided if smaller Adrenalin addition had been employed."

It is axiomatic that the less Adrenalin used, the less the danger. Wood[20] records the alarming increase in the rate of coronary disease in these islands, and also the lowering of the age group affected. The possibility of undiagnosed angina is remote, but he described one form which is accompanied by syncopal symptoms with loss of consciousness, so that the patient cannot appreciate pain, and therefore may be unaware of the gravity of his condition. In such a case, for instance, too much Adrenalin could easily have fatal results. But even without these rarer examples, surely it is common sense that the minimum dose which will provide adequate anesthesia, minimal side effects, motor nerve involvement, and minimum duration after completion of treatment, with its attendant accidental trauma to soft tissues, is the ideal dental requirement. It remains only to hint to the manufacturers that a 1.0 ml. cartridge would save considerable wastage and expense.

Table XI sets out exactly comparable work on each side of the mouth in 8 patients using procaine on one side, and the same dosage of Xylocaine on the other. The onset rates of between 2 and 3 to 1 are in conformity with the earlier figures. Lloyd and Blythe[14] compared the 2 drugs by bilateral injection in a similar way and found that, on average, 60 per cent of the procaine dose was adequate to produce the same result with Xylocaine.

In Table XII the data from 4 more patients were used in the same way as for Table XI, but the Xylocaine dosage was reduced in an attempt to find the equivalent which would produce the same result as procaine. The figures suggest that the Xylocaine equivalent is well under one-half of the procaine dosage.

TABLE XII
XYLOCAINE-PROCAINE EQUIVALENTS

NO.	ANESTHETIC	TYPE OF INJECTION AND AMOUNT (MENTAL)	ONSET		TREATMENT	EXTENT	DURATION (HOURS)	DEGREE
			LIP	OPERATIVE				
1.	Procaine	1.0 ml.	30″	5′	$\overline{3^c 4^c}$	2-6 (5)	2½	+4
	Xylocaine	0.5 ml.	30″	1′45″	$\overline{3^c 4^c}$	5-1 (5)	2	+4
2.	Procaine	1.0 ml.	1′	3′	$\overline{5^c}$	1-6 (6)	2	+4
	Xylocaine	0.5 ml.	45″	1′10″	$\overline{5^c}$	6-1 (6)	2¼	+4
		Infiltration	*Gum*	*Operative*				
3.	Procaine	1.25 ml.	30″	1′30″	$\overline{1^c 2^c 3^c}$	1-4 (4)	2½	+4
	Xylocaine	0.25 ml.	10″	1′45″	$\overline{2^c 3^c}$	2-4 (3)	2½	+4
4.	Procaine	2.0 ml.	15″	3′	$\overline{6^c 7^d 8^m}$	4-7 (7)	2½	+4
	Xylocaine	0.25 ml.	10″	2′15″	$\overline{5^d 6^m}$	3-6 (4)	2½	+4

CONCLUSIONS

It is no part of this work to deprecate the value of procaine, which should be regarded as the standard drug with which comparisons have been drawn. There has been complete unanimity in the literature about the superiority of Xylocaine to this standard, and it remains now only to suggest how best these advantages might be utilized in dentistry.

If the main advantages be briefly summarized under the various headings of the preceding discussion, the following information is clear.

		Procaine	*Xylocaine*
1. Operative onset time	Infiltrations	3′ 17″	1′ 21″
	Mental blocks	3′ 30″	1′ 42″
	Mandibular blocks	7′ 18″	4′ 30″
2. Extent	Infiltrations	4 units	5.5 units
3. Duration	Infiltrations	2 hr.	2½ hr.
4. Degree	All groups	30% failure	—
5. Effective dosage range	Infiltrations and mental blocks	1.5 to 2.0	0.5 to 1 ml.

(All figures are averages.)

Taking these statistical figures in conjunction with the individual case times analyzed, the information obtained in Tables XI and XII and the time-dosage graph, the author has postulated suitable dosages of Xylocaine for general practice which he believes are more suitable than those in general use, and which have been found adequate in every way by him in the course of well over 12,000 injections. They are as follows:

- 0.25 ml.
  - (1) Single teeth, especially upper premolars } Routine restorations
  - (2) Two adjacent upper premolars } Routine restorations
  - (3) Testing for cause of neuralgia (less if possible)
- 0.5 ml.
  - (1) Routine restorations } Upper { (a) molars; (b) canines; (c) one or two incisors
  - (2) Routine extractions } Upper { (a) molars; (b) canines; (c) one or two incisors
- 0.75 ml.
  - (1) Mental blocks
- 1.0 ml.
  - (1) Two-three adjacent upper molars (routine restorations)
  - (2) Single rooted teeth { Pulp extirpation; Jacket or ¾ crown preparations
  - (3) Infraorbital block
- 1.5 ml.
  - (1) Surgical extractions, except lower molars
- 1.5 to 2 ml.
  - (1) Mandibular blocks

The effect of the smaller dosage on duration and extent has already been referred to, while in regard to degree of anesthesia and onset time it is shown to be negligible. The smaller dosage should reduce possible toxicity.

SUMMARY

1. The clinical efficiency of 2 per cent Xylocaine with 1/80,000 Adrenalin has been compared with standard 2 per cent procaine/Adrenalin in a series of 329 injections.

2. Statistical data obtained from the 3 main groups (local infiltrations, mental blocks, and mandibular blocks) show that Xylocaine is superior to procaine in rapidity and onset, depth of anesthesia, and extent, and that duration is greater. It was further shown to be effective in very much lower doses than procaine.

3. The relationship between dose and site on one hand, and duration and extent on the other was investigated.

4. Confidence limits for Xylocaine dosages in all regions were established with 98 per cent confidence statistically, and graphs to indicate time-dosage relationships for both anesthetics were plotted.

5. Based upon these data, a series of doses lower than those in general use for everyday dental procedures has been put forward to reduce still more the danger of toxicity, and the duration and motor effects where necessary, avoiding mechanical damage to the tissues.

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PERIODONTAL FINDINGS IN CYANOTIC INDIVIDUALS

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IN HYPOXEMIA the organism attempts to improve function by establishing adaptative mechanisms. In the hemopoietic system formation of erythrocytes and hemoglobin is accelerated and in the tissue oxygen is better utilized, the capillary bed is dilated, and the permeability of the capillary endothelium is increased. By these processes, increase of the hemoglobin on the one hand and decrease of O_2 saturation in the circulating blood on the other hand, cyanosis that becomes manifest first of all in the acra and mucous membranes[1, 4, 6] is induced.

In chronic cyanosis clublike deformation of fingers and toes is induced. The anatomic and histologic structure of the ungual phalanx displays a striking similarity to that of the gingiva in that terminal arteries are found in both and both are devoid of subcutaneous (submucous) layers. For this reason the gingiva may be regarded as one of the acra and behaves like these. In diseases with acral lesions, gingival lesions are common. In Burger's disease (thromboangiitis obliterans), Raynaud's gangrene, endangiitis obliterans, scleroderma, and vascular disturbances of childhood (infantile acrodynia, acro-osteolysis, etc.) atrophy of the periodontium is a constant symptom.[3, 5, 8, 9] These diseases display, among others, the common symptom of acral cyanosis. In these diseases cyanosis is of peripheral origin, due to lesions of capillaries and arterioles. Cyanosis of central origin is seen, on the other hand, in cardiac insufficiency in persons with septal defects of the heart or by insufficient oxygenation of the blood in the lungs.

Periodontal findings were recorded in 2 groups of patients with cyanosis of central origin, viz. (1) in adults suffering acquired cardiac disease, and (2) in children suffering with congenital vitium.

One hundred and fifty-five adults, between 20 and 70 years of age, were studied. They had cardiosclerosis, valvular heart disease, carditis, or arterial hypertension. The duration of the cardiac disease varied between 2 months and 35 years. In 75 per cent of these patients alveolar atrophy was observed. The gingiva was rather livid in color, more cyanotic than inflamed. Atrophy of the gingiva and the alveolus occurs syncronously. No deep pockets are formed. In the majority of patients, where the atrophy was not complicated by intercurrent gingival inflammation, it started in the region of the lower first incisors and remained most marked in this region. Gingival atrophy of this region is characteristic first of all in young adults with otherwise intact dentitions (Fig. 1). The atrophy is aggravated by intercurrent gingivitis or

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occlusal trauma. With progress of age and of cardiac disease the severity and extension of periodontal atrophy increases. There were no marked differences as to the type of cardiac disease in relation to the alveolar atrophy. Since the duration of the cardiac disease increased with progressing age, these two phenomena could not be regarded separately (Table I).

Fig. 1.—Female of 21 years. Diagnosis: Vitium cordis decompensatum. Initial periodontal atrophy in the midline of the mandible.

Fig. 2.—Girl of 8½ years. Diagnosis: Fallot's tetralogy. Severe periodontal atrophy adjacent to the right upper deciduous canine and molars (not included in Table II).

Thirty-three persons suffering from congenital vitium were examined. Their ages ranged from 3 to 28 years. Twenty-five displayed cyanosis. In the 8 free of cyanosis the erythrocyte counts and hemoglobin values were near normal. In their gingiva no cyanosis, no inflammation, and no atrophy was observed. In some of this group rampant caries was observed, mostly due to excessive sugar intake. Among the 25 persons with cyanosis, atrophy was observed in 24. The gingivae are cyanotic, edematous, and swollen. The progress of atrophy is equal in the soft tissues and alveolar process, thus, clinically, the picture of periodontosis is seen without pockets. A hyperplastic gingivitis was found in one patient only. Gingival inflammation is rather moderate, being restricted to the marginal region. In some cases a severe atrophy is seen in the deciduous dentition (Fig. 2). In these cases,

TABLE I
OCCURRENCE OF PERIODONTAL ATROPHY BY PATIENTS SUFFERING ON ACQUIRED CARDIAC DISEASE ACCORDING TO AGE

AGE	20-30	30-40	40-50	50-60	60-70	
Number of patients	22	26	40	46	21	sum total: 155
Patients with atrophy (per cent)	50	70	83	80	87	average: 74.8

the atrophic process starts in the lower incisor region and progresses with age. In the stage of mixed dentition a more severe atrophy is seen in the region of the deciduous molars than about the permanent incisors, due to the more prolonged exposure of the former. When personal oral hygiene is neglected, soft debris is deposited on the denuded necks of the teeth. The degree of atrophy displays a parallelism with the degree of cyanosis. In normal childhood, especially in the deciduous dentition, periodontal atrophy is rare. Neither gingivitis nor occlusal trauma induces periodontal atrophy in normal children. It seemed worthwhile to examine the juvenile group more thoroughly, since, in children with cyanosis, periodontal atrophy is beyond any doubt related to their cyanosis. In contrast to the clublike thickening of the ungual phalanx in periodontium atrophy occurs. This different reaction in the 2 acral regions might be due to some local factors. In 15 patients of this group the kallikrein level in the saliva was examined. Kallikrein is a constant and stable organic constituent of the saliva, belonging to the group of the so-called H substances[2] and is found in all mammals. Kallikrein is produced by 2 types of glands, by the pancreas (associated with intact Langerhans islets) and by the salivary glands. Kallikrein is secreted on the one hand into the blood and, on the other hand, through the glandular ducts into the small intestine and oral cavity, respectively.

Like other H substances, kallikrein acts as a depressor of the blood pressure. The permeability of capillary walls is increased by kallikrein. It is found constantly in an inactive stage in the circulating blood. If injected intravenously, an abrupt fall of the blood pressure is induced but kallikrein becomes inactivated within a few seconds by a substance contained in the blood and the normal blood pressure is re-established. By this effect the kallikrein content of different body fluids may be measured. The salivary concentration is lower in young that in senile individuals. In periodontosis the kallikrein level of the saliva is uniformly high, irrespective of age.[7]

Of the 15 persons with congenital vitium, 4 displayed no or only a very slight cyanosis (Table II). In these 4 persons the gingiva was pale and not atrophic. The kallikrein level in the saliva was normal. In the saliva of persons with cyanosis a higher level of kallikrein was found than physiologically present, except in two patients. One patient (15) was in very desperate condition when examined and died within a few days. Patient 11, who had marked gingival hyperplasia, had approximately normal salivary kallikrein level in spite of the severe cyanosis.

TABLE II
THE KALLIKREIN CONTENT OF SALIVA BY PATIENTS SUFFERING CONGENITAL VITIUM

NO.	SEX	AGE	DIAGNOSES	CYANOSIS	ERYTHROCYTE COUNT (MILLIONS)	HGB. (GR. %)	HT.	BLOOD O_2 SATURATION	CLUBBED FINGERS	PERIODONTAL ATROPHY	KALLIKREIN IN SALIVA (U./ML.)
1	♂	15	Coarctation of aorta	None	5.0	14.5	—	—	None	None	2
2	♀	10	Tetralogy of Fallot	None	5.9	15.0	51	—	None	None	2
3	♂	26	Arteriovenous anastomosis	Very mild	5.0	14.5	—	—	Mild	None	1.5
4	♀	15	Tetralogy of Fallot	Very mild	6.4	19.0	—	66	Mild	+	1.5
5	♂	23	Eisenmenger complex	Mild	5.4	15.0	—	—	Mild	+++	2.5
6	♂	6	Mongolian idiocy	Mild	5.5	18.5	45	—	Mild	+	Over 6
7	♂	18	Tetralogy of Fallot	Strong	6.9	17.2	—	—	Strong	++	Over 6
8	♀	6	Tetralogy of Fallot	Strong	6.5	19.5	61	—	Mild	++	Over 6
9	♂	4	Tetralogy of Fallot	Very strong	6.9	22.5	—	67	Mild	++	Over 6
10	♀	3	Septal defect	Strong	6.8	23.0	70	64	Mild	++	Over 6
11	♀	20	Tetralogy of Fallot	Very strong	7.8	23.0	—	—	Strong	Hyperplasia	3
12	♀	28	Tetralogy of Fallot	Very strong	8.0	22.5	80	73	Strong	++	Over 6
13	♀	7	Tetralogy of Fallot	Strong	8.1	23.0	79	34	Mild	+	Over 6
14	♂	10	Situs inversus	Very strong	8.4	22.4	80	60	None	++	5
15	♂	9	Tetralogy of Fallot	Very strong	9.0	23.0	80	32	Mild	++	3

High salivary kallikrein levels are not unrelated to the gingiva. In histologic specimens of the gingiva in cyanotic children the lack of hornified epithelial layer was observed (Fig. 3). The connective tissue papillae were elongated and the surface of the epithelium covered by cells containing nuclei. Kallikrein may exert its vasodilatatory effect through the nonhornified gingiva and through slight traumatic lesions in the pockets. Similar to the increased permeability of the vascular wall in inflammation due to histamine-like substances and the migration of leukocytes into the tissues, an extrav-

Fig. 3.—Boy of 9 years. Diagnosis: Fallot's tetralogy (Pat. No. 15 in Table II). Cross section of the interdental papilla adjacent to the right upper deciduous canine.

asate is formed due to the action of kallikrein and the alveolar limbus becomes prepared for being destroyed. In the author's opinion a process similar to that in simple gingivitis occurs, but it is different in that it cannot be successfully managed by excluding the excitative factor. In patients with increased salivary kallikrein levels therapeutic attempts to reduce the gingival inflammation are frustrated since the increased salivary kallikrein level acts continuously as the exciting factor.

SUMMARY

According to the histologic structure and behavior under pathologic conditions the periodontium is to be regarded as one of the acra. In diseases of the other acra the periodontium is affected and, because of its special susceptibility, sometimes earlier than the others.

In peripheral vascular diseases pathologic changes are induced in the periodontium and in all other aera by the same exciting causes.

In patients with cyanosis of central origin the kallikrein level of the saliva is markedly increased. It is assumed that the long-lasting action of this increased kallikrein level in the saliva is contributory to the periodontal atrophy observed in these patients.

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A REPORT ON A RESIN COMPOSITION CAPABLE OF BONDING TO HUMAN DENTIN SURFACES

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IT IS generally known that presently available filling materials have no appreciable adhesion to tooth structure under oral condition.[1-4] Restorative materials capable of forming strong bonds to tooth structure would offer many advantages. Resistance and retention forms in cavity preparation would be less critical. Such a material could be used in sealing pits and fissures for caries prevention and in the repair of incisal fractures without the need for cutting tooth structure. The leakproof cementation of bridges and inlays would be possible. In order to realize these and additional advantages, we directed our efforts toward the development of an adhesive filling material. The results of one phase of our work are presented in this paper, wherein we report a series of in vitro experiments demonstrating a resin material capable of forming strong bonds to dentin surfaces.

EXPERIMENTAL

Qualitative.—In order that our laboratory tests might relate to oral conditions, the testing of materials for adhesion to tooth structure has been done on what we call "wet" specimens, i.e., dentin or enamel surfaces that have been stored in water and which, prior to use, are thoroughly wiped with an absorbent material and further dried with compressed air for several seconds. This method of drying we feel produces a condition of dryness comparable to what can be safely and easily attained with tooth structure, in vivo. In our initial tests we evaluated most of the available acrylic resin materials. In screening the materials for adhesion, we simply allowed a small amount of the resin mix to harden on a "wet" tooth surface. Adhesion was evaluated by noting the resistance offered by the material to removal by pressure applied with a sharp instrument. The results of these preliminary tests indicated that with one exception the acrylic resin materials had very little or no adhesion to "wet" dentin surfaces. The adhesion to enamel surfaces was excellent for all the resins tested but on water immersion the adhesion was destroyed within twenty-four hours. This increased adhesion is probably due to the better drying attainable with enamel as compared to dentin. The one material that seemed, at least qualitatively, to produce better adhesion was the Sevriton filling resin. Although better results were not consistently obtained, it was felt that there was sufficient improvement to warrant further study of this material.

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As commonly obtained, the Sevriton unit consists of methylmethacrylate monomer and polymer, a catalyst, and a liquid cavity seal. This latter material according to the manufacturer's directions is to be applied to the cavity surface prior to the insertion of the acrylic resin mix. The catalyst in the mix supposedly polymerizes the cavity seal liquid to a hard resin which adheres to the tooth surface. It is claimed that the cavity seal liquid contains a material having a special affinity for tooth structure which we have inferred from related patent literature to be a glycerophosphoric acid dimethacrylate. Our analysis of the cavity seal indicates that it contains 5 to 10 parts of the phosphoric acid ester, 5 to 10 parts of methacrylic acid, and 80 to 90 parts of methylmethacrylate with perhaps a trace of methacrylic anhydride. In our tests with this material we noticed that when good adhesion was obtained the surface of the dentin or enamel after removal of the hardened acrylic resin was always dry. Where poor adhesion was obtained the tooth surfaces after removal of the acrylic resin presented an oily appearance. We reasoned that the applied cavity seal liquid in the latter cases had not been fully polymerized because sufficient catalyst had failed to reach it from the superimposed acrylic mix. If, indeed, this was the case, all that was necessary to insure adhesion was to supply sufficient catalyst to the cavity seal liquid to cause its complete polymerization. This could be done by the direct addition of catalyst to the cavity seal liquid before it was applied to the tooth surface.

After some preliminary tests we selected a mixture that, when catalyzed, produced, in 5 to 8 minutes, a hard resin that formed a strong bond to "wet" dentin surfaces and which had excellent resistance to water immersion. The mixture consisted of the following ingredients mixed in the order given: (1) Sevriton cavity seal liquid 0.400 Gm.; (2) Methylmethacrylate polymer 0.040 Gm. plus 2 per cent benzoyl peroxide; (3) Methylmethacrylate monomer 0.100 Gm.; and (4) Sevriton catalyst 0.050 Gm. In place of the Sevriton catalyst we can use freshly prepared paratoluene sulfinic acid. Although the resin produced by the above mixture showed excellent adhesion to dentin, it had the disadvantage that it was quite brittle and gelled rather quickly, allowing relatively little handling time. Best results are obtained by placing the mix on the tooth surface just before it gels. Although the resin is insoluble in common solvents such as chloroform, acetone, alcohol, etc., it is slightly soluble in methylmethacrylate monomer. Consequently when an acrylic mix is applied over the adhesive resin, a good bond is established between the two materials. In this fashion conventional acrylic resins can be bonded to tooth structure using the adhesive resin as an interface.

Quantitative.—Since the acrylic resin materials could be bonded to the adhesive resin, we were able, by a method developed in our laboratory, to test the adhesion of the latter to the tooth structure. In these tests we used only flat dentin surfaces since enamel surfaces in the large amounts required were difficult to obtain. The surfaces were prepared in the following manner. Teeth were embedded in plaster blocks 1½ inches square and ground on a carborundum wheel of Grit No. 38A60-18VBE to expose a smooth

flat longitudinal surface of dentin surrounded by plaster. The block was then further ground on a silicon carbide wheel of Grit No. 37C80-MV to obtain a smoother surface. These blocks were stored in water until needed. When ready for use, the blocks were wiped with an absorbent material and the dentin surface further dried with compressed air for a few seconds. A ½ inch square in thin aluminum foil which had a 5 mm. diameter hole punched in the center was then fixed over the dentin surface with a small amount of mucilage. This left exposed an area of dentin corresponding to the hole in the foil to serve as the test surface.

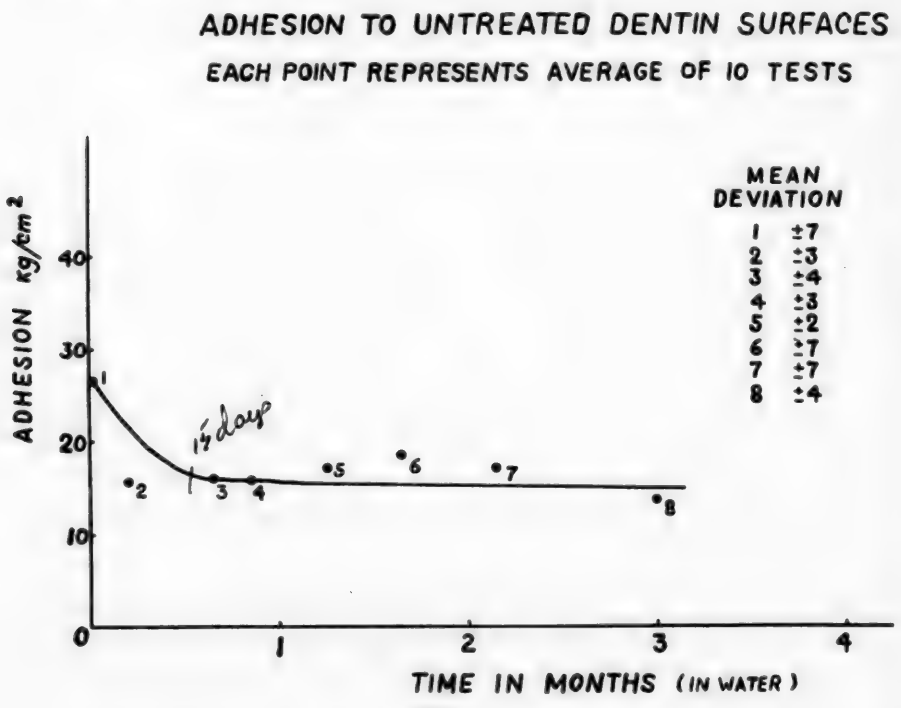

Fig. 1.

This area of dentin was then covered with a layer of the adhesive resin mix ranging in thickness from about 1 to 1½ mm., which was allowed to harden. Steel tubular matrices having only one open end, which was 1 mm. in diameter larger than the hole in the foil, were filled to slight excess with a mix of a standard acrylic resin filling material. The exposed part of the acrylic was then placed as concentrically as possible over the hardened adhesive and pressed into contact using finger pressure. Controls consisted of the acrylic resin similarly applied to dentin which had a prior application of a 1 to 1½ mm. layer of standard acrylic filling material instead of our adhesive. The acrylic resin was then allowed to harden and the assemblies placed in water until ready for tensile testing.

The adhesive strength of the bond was obtained by measuring the force in kg./cm.^{2}, applied at right angles to the dentin surface, required to separate the acrylic-matrix combination from the dentin surface. Readings were taken one hour after placing the assemblies in water and at intervals up to three months.

RESULTS

The results are shown in Fig. 1. No adhesion was obtained with controls. As is common in measurements of this type, we found that our results showed a fairly wide range of values. The percentage mean deviation increased in

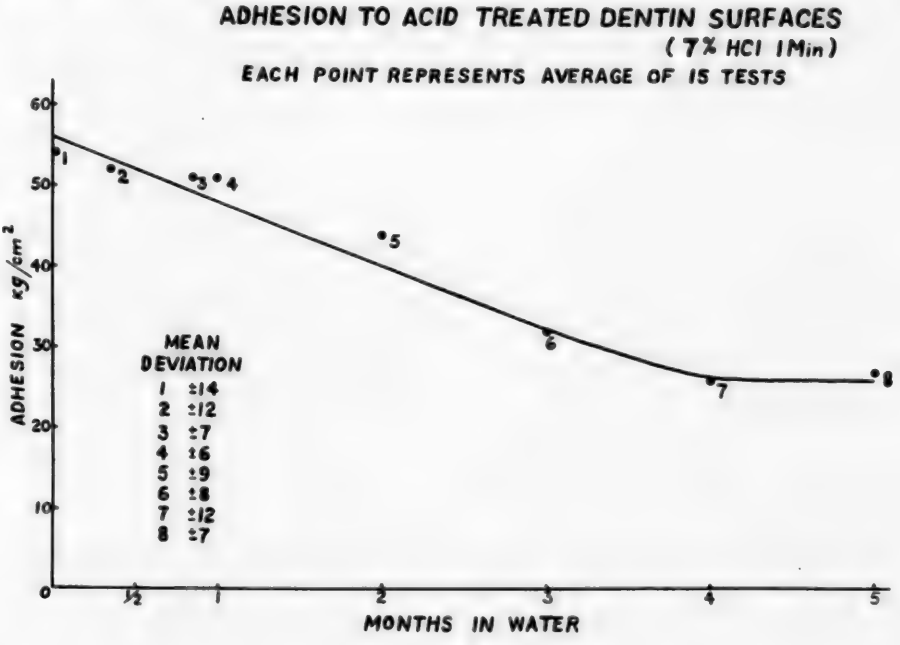

Fig. 2.

size with longer immersion times. The greatest loss in adhesion occurred during the first fifteen days after which time it seemed to level off. It was noted, when high adhesion figures were obtained, that a large portion of the adhesive remained attached to the dentin. This indicated that the break occurred between acrylic and adhesive or in the body of the adhesive layer rather than at the stronger bond between adhesive and dentin. In the majority of cases tested after one month in water, a clean break occurred between dentin and adhesive indicating that the bond with the dentin had been considerably weakened by water action.

Since the adhesive composition has a rather low pH, about 2.5, it was felt that it may have caused dissolution of the dentin surface and enlargement of the tubules. This condition would favor penetration of the adhesive mix or its components into the dentin, causing the adhesive to be mechanically

retained. It was thought that perhaps the adhesion of the standard acrylic resins applied to dentin surfaces might be similarly increased if these surfaces were previously acid treated. To test this hypothesis we treated dentin surfaces with one drop of a 7 per cent HCl solution for one minute prior to application of the standard acrylic resin filling material. We also decided to study the effect of this acid treatment on the adhesion of our adhesive resin.

The results are shown in Fig. 2. Acid treatment did not increase the adhesion of the ordinary acrylic resin. The adhesion, however, obtained with our adhesive material applied over acid-treated surfaces is about double that obtained on untreated dentin. In most of the cases, tested during the first two months, large portions of the adhesive remained bonded to the dentin. With the higher readings obtained during the first month, usually 90 per cent of the surface was covered with the adhesive material. The loss of adhesion to the treated surfaces seems to be more gradual than with the untreated ones.

DISCUSSION

In evaluating the results, we wish to emphasize that they are purely laboratory findings obtained under conditions that may not apply in the mouth. To date we have studied extensively only the effect of water immersion on the adhesive bond. While the bond has given a good performance on water immersion, this is only the first phase of its evaluation. On the basis of other preliminary qualitative tests, however, the outlook for the bond is favorable. Many of the adhesive-dentin assemblies have withstood boiling water for as long as one hour after which considerable manual force was required to dislodge the adhesive. The bonds seem to hold up quite well when subjected to hot water, cold water interchanges for one hour. The results of twenty-four-hour dye penetration studies are also quite encouraging in that the dentin covered by adhesive is not stained although the resin itself picks up dye. It is our opinion that the glycerophosphoric acid dimethacrylate is the effective bonding agent in the cavity seal. The phosphate group in this compound seems ideally suited for chemical combination with constituents of the dentin. The role of the methacrylic acid in establishing adhesion, however, cannot be discounted, and we are experimenting with this material to determine its function.

With respect to the mode of action of our adhesive, preliminary histologic studies of the bonded area show a marked alteration in the staining reactions of the dentin layers adjacent to the adhesive. This lends support to the idea that chemical reaction may have occurred between the organic matter of the dentin and the constituents of the cavity seal. If, indeed, chemical combination can be demonstrated as a basis for the adhesion observed, it will represent a significant development in our attempts to bond resin materials to tooth structure.

It is felt that our approach to the problem of bonding resin filling materials to tooth structure is a realistic one and that the results obtained show the possibility of bonding these materials to dentin under oral conditions. More

important than any practical clinical aspects at this time is the possibility suggested by our results that a certain chemical structure is required in order to obtain adhesion to dentin. If this point can be established, then compounds can be specifically tailored for the job of adhesion.

SUMMARY

1. We have demonstrated an adhesive acrylic resin material that bonds strongly to "wet" dentin surfaces of extracted teeth. The amount of adhesion can be approximately doubled by an acid etching of the dentin surface prior to application of the adhesive resin.

2. The bonds show good resistance to water immersion. For the untreated surfaces, initial bond strengths of 28 kg./cm.^{2} are reduced to 15 kg./cm.^{2} after three months water immersion. Initial values for acid-treated surfaces of 53 kg./cm.^{2} are reduced to 28 kg./cm.^{2} after five months water immersion.

3. The possibility is suggested that the bonding is due to chemical combination between one of the constituents of the seal and the organic matter of the dentin.

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EXPERIMENTAL OBSTRUCTIVE ADENITIS IN THE MOUSE

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INTRODUCTION

EXPERIMENTAL obstruction of the excretory duct of submaxillary and sublingual salivary glands of mice was produced for the following reasons: (1) It is believed that the mucoceles are caused by an obstruction of the excretory ducts and are retention cysts lined by epithelium.[1] It was therefore hoped that experimental ligation would shed some light on the pathogenesis of mucoceles; (2) The main cause of the inflammatory changes in the major and minor salivary glands is believed to be an ascending infection from the oral cavity or less frequently an infection via a hematogenous route. However, partial or complete obstruction of the excretory duct has also been claimed as a contributory factor. Experimental obstruction of the duct was produced to determine whether obstruction per se can lead to adenitis; (3) A study of human obstructive adenitis showed a great variety of histologic pictures. This was interpreted to be due to the difference in the duration of obstruction in different cases. It was hoped that the present experiment would establish the sequence of events which would explain the variety of histologic features seen in human obstructive adenitis.

REVIEW OF LITERATURE

The effect of ligation on the salivary glands has been studied in dog,[2-8] rabbit,[9, 10] guinea pig,[11] mouse,[12-14] rat,[15] and man.[5, 16-18] Although the duration of experiment and the number of subjects varies greatly, the results of these experiments may be summarized as follows: (1) Ligation leads to dilatation,[3, 5, 8-10, 16] atrophy,[12, 13, 16] or proliferation[4, 11] of the ducts; (2) The parenchymal cells degenerate,[9-11] atrophy,[2, 10, 12, 13, 15, 16] or decrease in amount;[3] (3) The interstitial tissue shows neutrophilic or lymphocytic or other cellular infiltration[2, 3, 5, 9, 10, 16] and fibrosis.[4, 8, 11] Since a number of these studies were attempted either to establish an endocrine function of the salivary glands[6, 9] or to control diabetes[5, 7, 17, 18] the histologic description of the affected glands is either omitted[6, 7, 18] or not given in detail. With the exception of one study in man where the ligation lasted for 10 months in 3 subjects, the longest postoperative experimental period reported is 113 days,[8] and the majority of studies extended from 7 to 90 days.

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In none of the investigations has the formation of lesions comparable to human mucoceles been reported. In spite of this fact it is surprising that the mucoceles are still considered to be retention cysts.

MATERIAL AND METHODS

Thirty-one 6-month-old female mice were anesthetized with ether, the submandibular regions were shaved, and the submandibular groups of salivary glands exposed by a skin incision. The right submaxillary duct was then dissected out from the surrounding tissues and ligated. The covering skin was sutured. Animals were sacrificed at 1 (2), 2 (2), and 3 (2) weeks, and at 1 (2), 2½ (1), 3 (2), 4 (3), 4½ (1), 5 (3), 5½ (2), 7 (2), 9 (2), and 9½ (7) months postoperatively.* The recovered glands of the right side were fixed in 10 per cent formalin and embedded in paraffin, cut serially and stained with hematoxylin and eosin, Van Gieson, Mayer's Mucicarmine, and erythrosin-safranine. The glands from the left side were treated similarly except that they were not cut serially.

FINDINGS

Normal Submaxillary and Sublingual Glands of the Mouse.—The submaxillary and sublingual glands lie in a common capsule and are glands of special serous and mucous types, respectively. There is no agreement whether these glands have a common duct or separate excretory ducts. The excretory system starts with the intercalated ducts which empty into the intralobular and finally into the interlobular ducts. The latter join to form the major duct.

In the *submaxillary* gland the major duct and the interlobular ducts are lined by a single layer of cuboidal epithelium, which has eosinophilic cytoplasm and a round, centrally placed nucleus. The intralobular ducts are numerous (Fig. 1). They are lined by pyramidal or columnar epithelium, the coarsely granular cytoplasm of which is deeply eosinophilic. The round or ovoid nuclei are basally placed, the cellular outlines, due to coarse granules, are not distinct, and the luminal surface of the cells appears frayed (Fig. 1). The lumina of the intralobular ducts are narrow. The intercalated ducts are short and, therefore, in histologic sections are seen infrequently. They are lined by low cuboidal cells, the cytoplasm of which is homogeneous. The acini are composed of pyramidal cells with vacuolated basophilic cytoplasm and a round, basally placed nucleus. Lumina of the acini can hardly be recognized. Under low power the lightly basophilic acini lie between the abundant, more striking eosinophilic intralobular ducts. Mucicarmine stained the acinar cells red (Fig. 2), and the lining cells of the intralobular ducts very pale pink. The intercalated ductal cells did not stain. Delicate interstitial connective tissue intervenes between the acinar and ductal elements.

The *sublingual* gland, as compared to the submaxillary gland, shows fewer and less striking intralobular ducts. All ductal elements of the mucous gland are lined by eosinophilic cuboidal epithelium, the cytoplasm of which is agranular. The striking feature of this gland is its numerous acini, which consist of

*The figures in parentheses indicate the numbers of animals in each group.

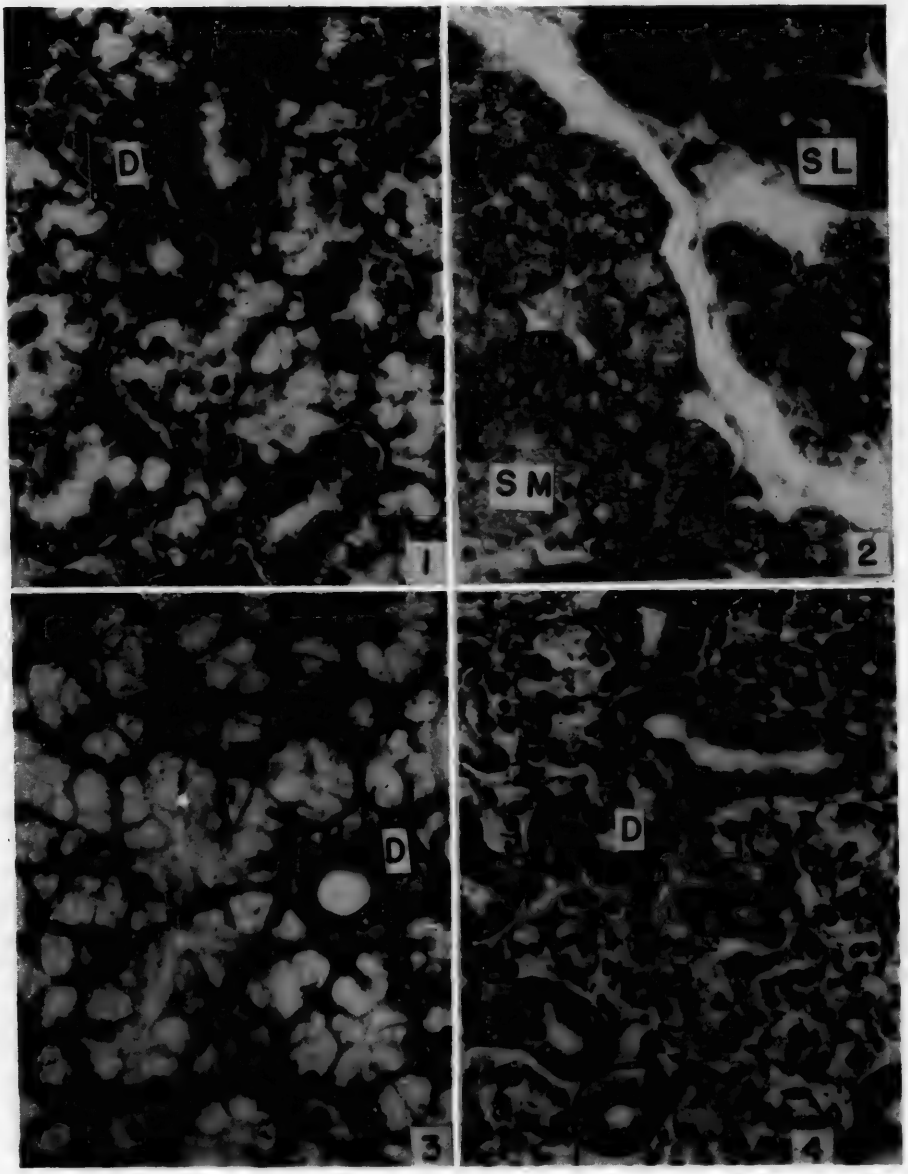

Fig. 1.—Normal submaxillary gland. Note numerous intralobular ducts (*D*). Acinar cells intervene between the ducts. Hematoxylin and eosin, orig. mag. ×600, reduced ⅙.

Fig. 2.—Photomicrograph of a section stained with mucicarmine. Note that both the submaxillary (*SM*) and sublingual (*SL*) acinar cells are mucine-positive. The latter, however, stain more intensely. Mucicarmine, orig. mag. ×600, reduced ⅙.

Fig. 3.—Normal sublingual gland. Note intralobular duct (*D*). Orig. mag. ×600, reduced ⅙.

Fig. 4.—Submaxillary gland one week after ligation. Intralobular ducts (*D*) are outlined. Acinar cells are reduced in size. Hematoxylin and eosin. Orig. mag. ×600, reduced ⅙.

large, pyramidal, intensely vacuolated cells with basally placed flattened nuclei (Fig. 3). Lumina of the acini are not apparent. Acini are separated by a very delicate connective tissue stroma.

Mucicarmine stains the mucous acini deep red while the cells lining the duct stained very pale pink. The mucous cells were stained darker than the cells of the submaxillary gland.

Submaxillary and Sublingual Glands on the Left (Control) Side.—The interstitial connective tissue of the submaxillary and sublingual glands on the control side showed lymphocytic and plasma cell infiltration which disappeared from 2 to 3 weeks postoperatively. In all other respects the glands on the left side appeared normal.

Submaxillary and Sublingual Glands on the Right (Experimental) Side.—

One week: A week after the ligation of the duct the most striking change in the *submaxillary* gland was the disappearance of the eosinophilic granules of the cells of the intralobular ducts. The pyramidal cells of these ducts now appeared cuboidal and had distinct boundaries (Fig. 4). The diameter of the intralobular ducts was reduced but the interlobular ducts appeared dilated and lined by a single layer of short cuboidal cells. The acini could no longer be seen distinctly, but appeared as small clusters of polyhedral vacuolated basophilic cells with round or oval nuclei (Fig. 4). These cell groups were crowded between the more apparent tubules and ducts. The periglandular, as well as the interstitial connective tissue of the gland, showed diffuse infiltration by neutrophils, some plasma cells, lymphocytes, and macrophages.

The *sublingual* gland showed a dilatation of the interlobular ducts but no significant change in the intralobular ducts. The acini as well as their component cells were smaller in size (Fig. 5), the cellular outlines were more distinct, and in some places the lumina could now be identified. The latter was due to the reduction in the number of secretory vacuoles and the size of the acinar cells. The cells, however, were still moderately vacuolated and showed basophilia. The periglandular as well as the interstitial connective tissue showed diffuse infiltration by neutrophils, plasma cells, and macrophages.

Two weeks: In the submaxillary gland 2 weeks after ligation there was a further dilatation of the interlobular and of some intralobular ducts. Many of the former appeared tortuous and in tangential sections cystlike (Figs. 6, 7, and 8). These ducts were lined by flattened epithelium. The prominent picture of the submaxillary gland, however, was a large number of small tubular structures which were lined by cuboidal epithelium. In between these ductal elements the once acinar cells appeared in one of the three forms. Either they formed small clumps of rounded basophilic vacuolated cells which resembled normal cells except in size, or they appeared as islands of signet ring cells with one large vacuole and crescent-shaped nuclei (Fig. 9), or they formed small masses of polyhedral cells with scanty nonvacuolated cytoplasm and relatively large, round nuclei. These three types, respectively, seemed to represent different degrees of decreasing activity and final rest. Degeneration of cells

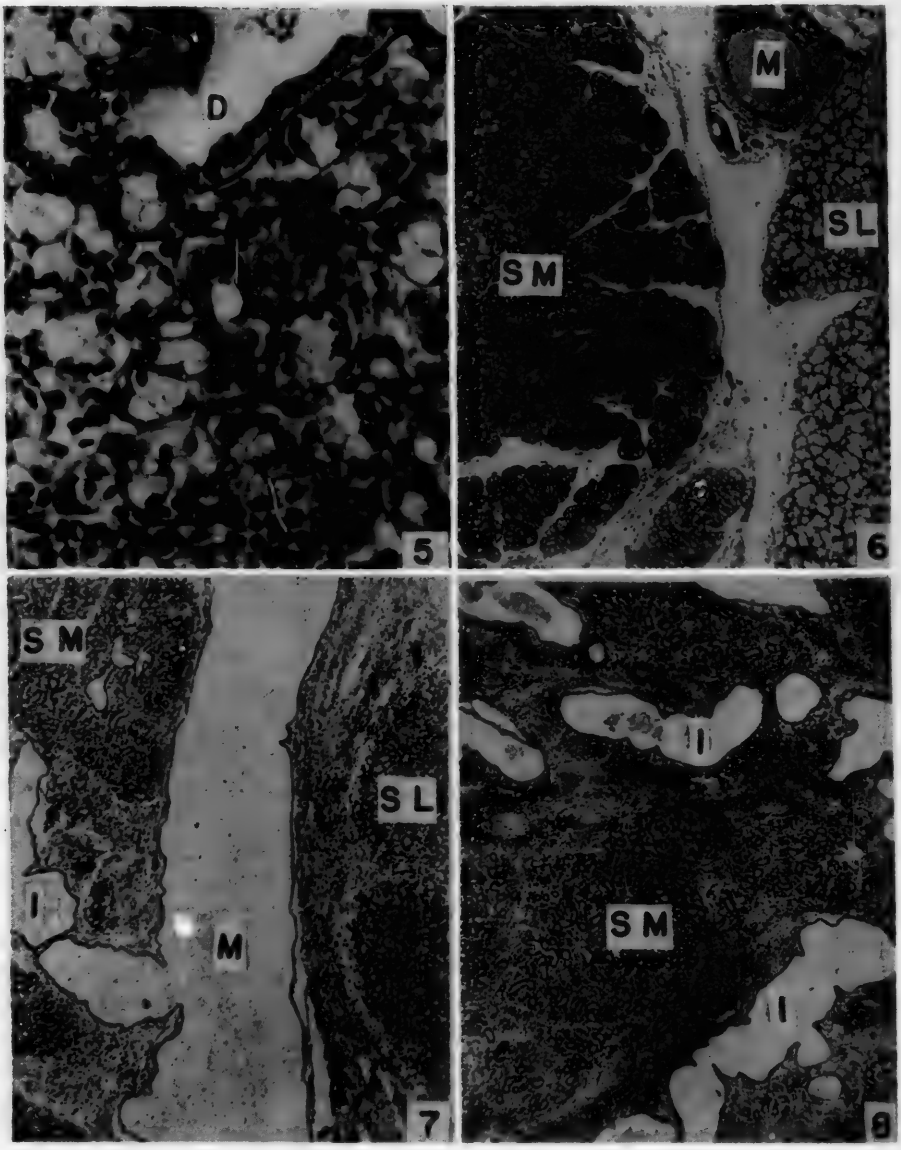

Fig. 5.—Sublingual gland one week after ligation. *D*, intralobular duct. Compare with Fig. 3. Hematoxylin and eosin. Orig. mag. ×600, reduced ⅙.

Fig. 6.—Normal sublingual (*SL*) and submaxillary (*SM*) glands. Note the major duct (*M*).

Figs. 7 and 8.—Sublingual (*SL*) and submaxillary (*SM*) glands 2 weeks after ligation. Note dilated and tortuous major (*M*) and interlobular (*I*) ducts. In Fig. 8 the submaxillary gland seems to consist predominantly of ducts. Hematoxylin and eosin. Orig. mag. ×85, reduced ⅙.

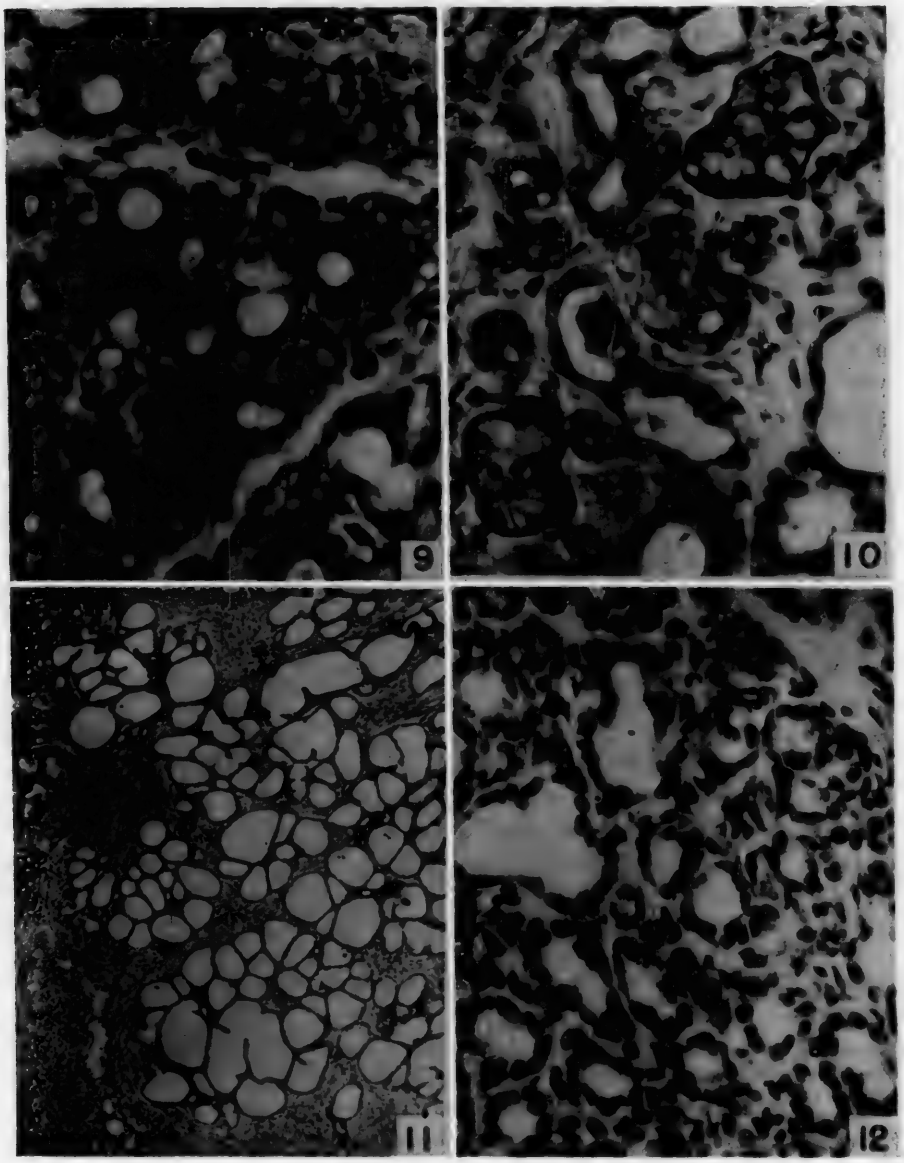

Fig. 9.—Submaxillary gland 2 weeks after ligation. Note numerous clumps of signet-ring cells which intervene the intralobular ducts. Hematoxylin and eosin. Orig. mag. ×600, reduced ⅓.

Fig. 10.—Submaxillary gland 4 months after ligation. Outlined areas show the clumps of resting acinar cells. Hematoxylin and eosin. Orig. mag. ×600, reduced ⅓.

Fig. 11.—Submaxillary gland 2½ months after ligation. Note numerous dilated intralobular ducts. Hematoxylin and eosin. Orig. mag. ×85, reduced ⅓.

Fig. 12.—Submaxillary gland 4 months after ligation. Note duct proliferation. Hematoxylin and eosin. Orig. mag. ×600, reduced ⅓.

was also noted in a few areas. The periglandular and interstitial inflammatory infiltrate was less marked and consisted predominantly of plasma cells and lymphocytes.

The ductal elements in the *sublingual* gland were similar in structure but markedly less numerous than those of the submaxillary gland. The acini were less numerous and smaller than in the normal gland. However, as compared to the ligated submaxillary gland, they were more numerous. They consisted mainly of rounded vacuolated basophilic cells, but in some instances the cells had lost the vacuolation and had become smaller and polyhedral. Some areas of cellular degeneration could be seen.

Three weeks: During this period further changes were observed in the *submaxillary* gland. The acinar cells which previously could be differentiated from the tubular cells became inconspicuous. Only in isolated areas could clumps of polygonal cells be identified.

The *sublingual mucous* gland appeared unchanged from the previous stage and was conspicuous because of the relative predominance of acinar cells.

One to four months: In the *submaxillary* glands the acinar cells continued to show regressive changes. They appeared as islands of nonvacuolated or lightly vacuolated polyhedral cells (Fig. 10). In some areas the intralobular ducts formed sprouts which consisted either of nonvacuolated or vacuolated cells. When vacuolated they resembled acinar cells but were mucin negative.

There was a progressive increase in the diameter of the intra- and interlobular ducts (Fig. 11). The lining cells of these dilated ducts varied from low cuboidal in some intralobular ducts to very flattened scalelike cells of the interlobular ducts. The cuboidal cells of some intralobular ducts showed faint eosinophilia.

The prominent feature at 4 months was the proliferation of ductal elements (Fig. 12). Mitoses were seen in the lining cells and frequently small sprouts could be seen originating from the ducts. Many areas, particularly the peripheries of the gland, showed small clumps of cells which were not yet canalized and differed from the islands of acinar cells by their small size and more regular arrangement.

From 1 to 4 months postoperatively the *sublingual* gland showed a progressive decrease in the number of mucin-positive acinar cells until at 4 months only a few remained. However, there was a progressive increase in the number of ducts. Ductal proliferation could be seen especially well 4 months after ligation. New ducts arose as sprouts of old ducts or could be seen as small uncanalized circumscribed islands of cells. In some areas of the mucicarmine-stained specimens, some of the cells lining the ducts showed ballooning and positive staining with mucicarmine (Fig. 13). Thus the formation of new acinar cells from ductal cells could be demonstrated.

Throughout this period the two glands showed interstitial infiltration of plasma cells. At 4 months after ligation a slight increase in the interstitial fibrous connective tissue could be observed.

Five to nine and one-half months: In the *submaxillary* gland from 5 to 7 months after ligation the predominant number of acinar cells appeared as clumps, cords, or islands of polygonal cells with scanty cytoplasm and relatively large nuclei. At some places they were continuous with ductal cells and could be differentiated from them mainly by the absence of lumina. At 9 and 9½ months after ligation some of the acinar cells again showed vacuolation or the

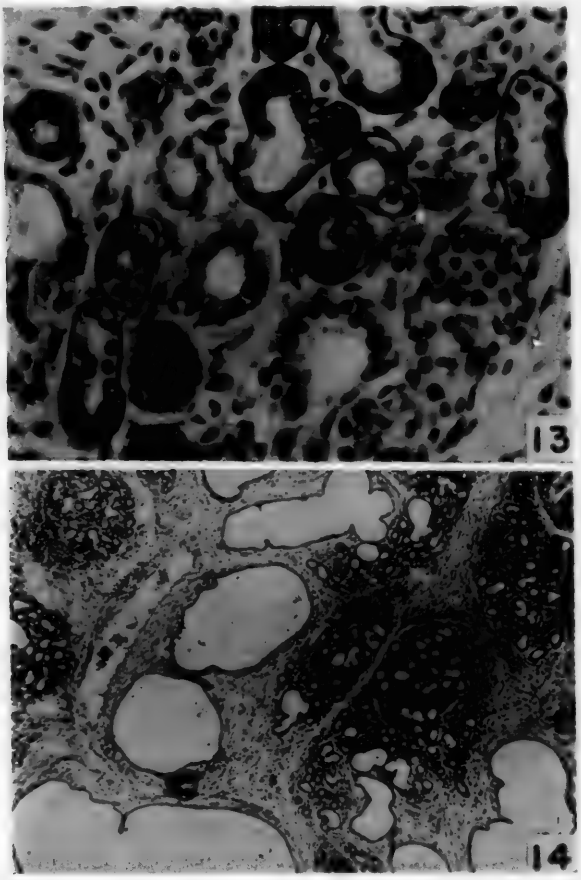

Fig. 13.—Sublingual gland 4 months after ligation. Mucicarmine stain. Note mucin-positive cells differentiating from mucin-negative cells of the ducts. Orig. mag. ×600, reduced ⅓.

Fig. 14.—Submaxillary gland 9½ months after ligation. Note fibrosis, areas of duct proliferation, and cystlike spaces which represent tangential cuts through dilated, tortuous, interlobular ducts. Hematoxylin and eosin. Orig. mag. ×85, reduced ⅓.

signet-ring appearance. From 5 to 9½ months there was a progressive increase in the lumina of all ductal elements so that even the smallest intralobular ducts appeared larger than in earlier stages. In many places large "cystlike" empty spaces always lined by flattened epithelium could be seen (Fig. 14).

Study of serial sections revealed that these cystlike spaces represented tangential cuts through dilated tortuous ducts. Some interlobular ducts showed patches of stratified squamous epithelium. There was a progressive increase in interstitial fibrous connective tissue, so that the intralobular ducts appeared farther apart from each other. In some cases fibrosis was particularly marked around the interlobular ducts (Fig. 14).

Between 5 and 9½ months the *sublingual* gland resembled the submaxillary except that it showed a number of acini of mucous cells and the presence of eosinophilic material in some of the ducts. The ductal elements though more numerous than earlier were lesser than in the submaxillary gland.

The interstitial tissue of these glands showed a progressive decrease in inflammatory cells, but even at 9½ months after ligation it showed lymphocytic and plasma cell infiltration.

DISCUSSION

1. *Pathogenesis of Mucoceles.*—Mucoceles are grossly visible, circumscribed, translucent swellings. It is generally accepted that they are retention cysts which follow an obstruction in the salivary ducts.[1] Histologically they are described to be epithelium-lined cavities which contain mucous, inflammatory, and desquamated cells. In the present experiment where the obstruction of the excretory duct lasted from 6 days to 9½ months mucoceles did not develop. Histologic sections did reveal epithelium-lined "microcysts" which represented tangential cuts through the tortuous dilated ductal elements. It can be concluded from this study, therefore, that mucoceles are not necessarily caused by obstruction of excretory ducts of salivary glands. This view is further strengthened by the fact that in none of the previous investigations where the salivary ducts were ligated could the formation of mucocele-like lesions be demonstrated.

2. *Duct Obstruction and Adenitis.*—Inflammation of the human salivary glands is supposed to be due either to an ascending infection from the oral cavity or more rarely via a hematogenous route. It has been suggested that the obstruction of the duct as in sialodochitis or sialolithiasis may contribute to interstitial inflammation of the gland.[1, 19] In the present study, whereas both the control and experimental sides were subjected to similar surgical manipulation, the inflammatory changes on the control side disappeared by about the fifteenth day, whereas on the experimental side chronic adenitis was noticed even 9½ months after ligation. From this it may be concluded that ligation per se, in the absence of any superimposed infection, can cause and maintain chronic inflammation. It is quite possible that the degeneration of cells which occurs following ligation leads to the production of some substance which, in turn, is responsible for the inflammatory response.

3. *Experimental and Human Obstructive Adenitis.*—The changes following experimental ligation and those seen in surgically removed human cases of obstructive adenitis are comparable. The detailed changes in human obstructive adenitis will be reported elsewhere. It may be briefly mentioned, however, that, whereas early cases show dilatation of ducts, degeneration of acinar

cells and interstitial infiltration by plasma cells and lymphocytes, in cases with longer histories there was duct proliferation and progressive fibrosis. In one specimen with a history extending over 36 months the histologic picture superficially resembled a fibrocystadenoma.

4. *Common Sublingual and Submaxillary Duct.*—Whether the sublingual and the submaxillary glands in mice have a common or separate excretory duct has been a matter of dispute (Sreebny).[20] Since in the present study the ligation of the submaxillary duct led to changes in both of these glands it can be assumed that they share a common duct.

5. *Cellular Changes.*—The primary effect of ligation as expected is upon the secretory cells. These include the mucous and serous acinar cells and the cells lining the intralobular ducts of the submaxillary gland. As a consequence of ligation, the acinar cells, both serous and mucous, become markedly reduced in size, showed decreased vacuolation, appeared signet-shaped, and, finally, avacuolated and undistinguishable from the altered ductal cells. Contrary to a previous report,[12] we also noticed signs of cellular degeneration. When this occurred in most of the cells of an acinus, microcysts formed. In addition to degeneration, sprouting of the intralobular ducts was noticed. These sprouts generally consisted of avacuolated cells, but in places some of these cells showed vacuoles. Differentiation of ductal cells into acinar cells was particularly noticeable in the sublingual gland, where mucin-negative cells of the ducts showed buds and demilunes of mucin-positive mucous cells. Thus the ductal elements were differentiating into new acinar cells.

The pyramidal cells lining the intralobular ducts of the submaxillary glands are also secretory and, therefore, markedly affected by ligation. They lost their granules, became markedly reduced in size, and appeared cuboidal. In later stages due to dilatation of some of the intralobular ducts, the lining cells became flattened.

In both glands there was an increase in the number of ducts. This indicates that the ductal cells are not only the source of the acinar cells, but also of new ducts. This ability of the ductal cells is also evidenced during development where the entire acinar component of the gland originates from the ducts. These findings also add further proof to the belief that, in the varied tumors of the salivary glands, it is the pleuripotential ductal cell which proliferates into various cell types.

SUMMARY

To study the relationship between duct obstruction on one hand to mucoceles and adenitis on the other, right submaxillary ducts of 31 6-month-old female mice were ligated. Animals were sacrificed at 1, 2, and 3 weeks, and at 1, 2½, 3, 4, 4½, 5, 5½, 7, 9, and 9½ months postoperatively. Glands from the right side were fixed in 10 per cent formalin, embedded in paraffin, sectioned serially, and stained with hematoxylin and eosin, Van Gieson, Mayer's Mucicarmine, and erythrosin-safranine. The left glands were treated similarly except that they were not cut serially.

The findings and conclusions were: (1) Lesions resembling human mucoceles did not follow duct obstruction; (2) Ligation per se causes adenitis; (3) Changes in the gland are comparable to those following obstruction of salivary ducts in man; (4) Ductal cells are the source of new ducts as well as nonfunctioning or partially active acinar cells; (5) Following ligation the secretory cells degenerate or show loss of secretory granules and vacuoles and finally appear as cuboidal or polyhedral cells; and (6) Ligation leads to increase in number of ducts as well as interstitial fibrosis.

We are indebted to Stefan Chobanoff for excellent technical assistance. The help given by William Winn, biological photographer, in the preparation of photomicrographs is gratefully acknowledged.

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PATHOGENESIS OF MUCOCELES

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INTRODUCTION

IT IS widely accepted that mucoceles are retention cysts which are lined by epithelium and result from the obstruction of a salivary duct.[1] In a previous experiment,[2] however, where the salivary ducts of the submaxillary and sublingual gland in mice were ligated, mucoceles did not develop. The present study was therefore undertaken to determine the pathogenesis of mucoceles. It consists of two parts. In the first part the histopathology of human mucoceles based on 19 cases is described. In the second part experimental production of mucoceles is reported. Finally conclusions as to the pathogenesis of these lesions are drawn.

REVIEW OF LITERATURE

There are only few reports on the pathogenesis or histopathology of mucoceles or ranulae. It has been generally accepted that the mucoceles are retention cysts, i.e., they are caused by an obstruction in an excretory duct.[1] Hipple[3] reported that the ranula is an epithelial-lined cyst which results from the retention of secretion. The obstructed dilated ducts fuse and form a large cystic cavity. Suzanne (quoted by Hipple[3]), Bailey,[4] and Brunner[5] on the other hand showed that ranulae, at least some of them, are a result of myxomatous degeneration or liquefaction of the mucous glands.

Descriptions as to the morphology of the mucoceles and ranulae also vary. Thoma[1] and Suzanne[4] have reported an epithelial lining, while Hipple,[3] Erdheim,[6] and Lang[7] emphasized that the cyst wall may be covered by epithelium-like cells but regular epithelium is never present. Brunner[5] described a case of ranula where an epithelial lining was missing.

MATERIAL AND METHOD

Part I.—The material consists of 19 surgically removed mucoceles. The removed specimens were fixed in 10 per cent formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin, Mayer's Mucicarmine, silver impregnation, Mallory's connective tissue stain, and Van Gieson's method.

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Part II.—To produce mucoceles experimentally five 6-month-old female mice and six 3-month-old female rats were anesthetized with ether, and after shaving the skin of the neck, the submandibular group of salivary glands was exposed by a skin incision. The right submaxillary excretory duct was then dissected out of the surrounding tissues and severed. The animals were sacrificed at intervals of from 1 to 9 days postoperatively. After sacrifice, the glands were exposed, studied grossly, removed, fixed with 10 per cent formalin, embedded

Fig. 1.

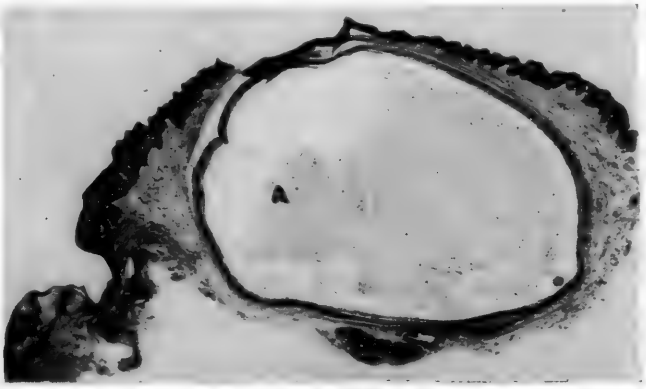
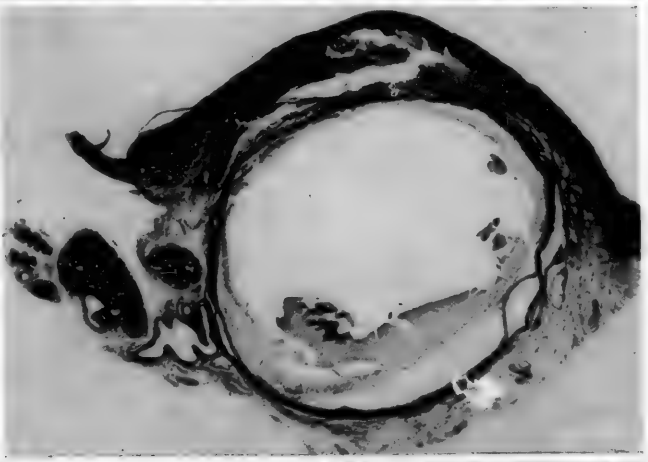


Fig. 2.



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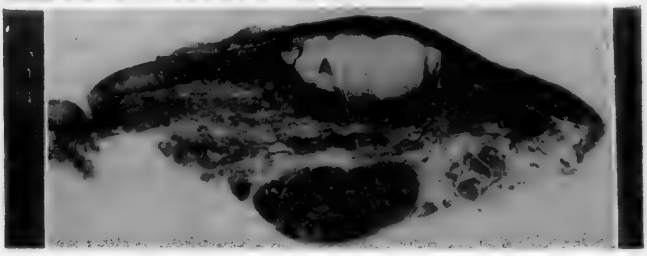


Figs. 1, 2, and 3.—Photomicrographs of typical human mucoceles (type 1). Note the well-circumscribed cystic cavities and the pale stained mucicarmine-positive contents (*A*). Stained with hematoxylin and eosin. Orig. mag. ×12, ×16, ×17, respectively, reduced ⅓.

in paraffin, cut serially, and stained with hematoxylin and eosin, Mayer's Mucicarmine, silver impregnation, Mallory's connective tissue stain, and Van Gieson's method.

FINDINGS

A. *Human Material.*—Of the 19 cases of mucoceles included in this study 11 originated in the lower lip, 2 at one of the commissures, 2 on the buccal mucosa, 2 on the ventral surface of the anterior part of the tongue, 1 in the floor of the mouth, and in 1 the location was not given. According to the patients the lesions had persisted for a period ranging from 3 weeks to 1½ years. Cases of longer duration reported intermittent swellings, which, after spontaneous drainage or incision, always recurred.

These lesions presented 1 of the 2 following histologic pictures:

Type 1: In most of the specimens the mucoceles appeared as distinctly circumscribed cavities which involved the lamina propria and the submucosa of the mucous membrane and produced an elevation on the mucosal surface (Figs. 1 to 3). The stratified squamous epithelium of the oral mucosa which covered this raised area was in all cases reduced in thickness and had lost its ridges. The cystic cavities, when intact, were filled with a homogeneous, vacuolated, eosinophilic, mucin-positive material in which varying amounts of cells were embedded. The embedded cells were spherical. From the shape of their nuclei at least some of these could be recognized as polymorphonuclear leukocytes and macrophages. In none of the examined specimens could a distinct epithelial lining be demonstrated. Silver staining showed that argyrophilic fibers extended to the border of the cystic cavity (Fig. 4). Sections stained with Mallory's connective tissue stains also revealed that the border of the cystic cavities was formed by connective tissue. The cyst wall consisted either of compressed connective tissue with varying degrees of lymphocytic infiltration, or, as was more often the case, of a wide band of granulation tissue (Fig. 5). The latter showed edema, mild lymphocytic and plasma cell infiltration, and abundant young fusiform fibroblasts with pale ovoid nuclei (Fig. 6). The cells closest to the lumen showed ballooning and desquamation. In some specimens where an adjoining mucous gland was also removed with the cyst, atrophy of the acini, interstitial fibrosis, lymphocytic and plasma cell infiltration, and dilatation of ducts could be seen in the gland.

Type 2: Five of the specimens, which were clinically diagnosed as mucoceles and which were of relatively short duration, did not show distinct, circumscribed cavities (Figs. 7 and 8). However, they produced raised areas on the mucosa. The covering epithelium showed thinning and disappearance of epithelial ridges (Figs. 7 and 8). The lamina propria and the submucosa showed varying amounts of intercellular, homogeneous, and in some areas fibrillar, mucicarmine-positive material as well as empty spaces (Fig. 9). In parts of the specimen the homogenous material reached up to and invo[illegible]d the basement membrane of the covering oral epithelium. Tissue surrounding these pools of mucin showed neutrophil and lymphocytic infiltration and some areas of fibroblast proliferation.

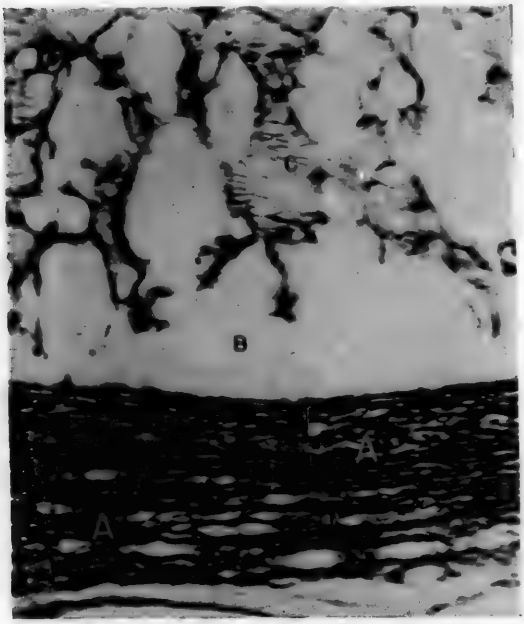
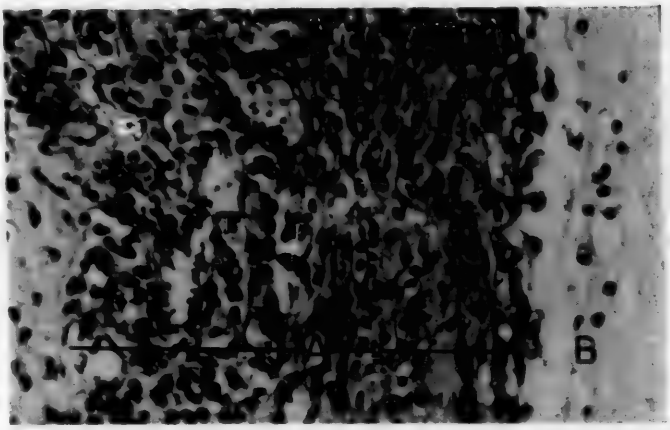


Fig. 4.

Fig. 5.

Fig. 4.—Photomicrograph of wall of mucocele shown in Fig. 1. Note the extension of the argyrophil fibers (*A*) to the surface of the cavity (*B*), and the coagulated mucous content of the cyst (*C*). Silver impregnation. Orig. mag. ×290, reduced ⅛.

Fig. 5.—Photomicrograph of wall of mucocele (Fig. 2) showing wide layer of granulation tissue (*A*) infiltrated by inflammatory cells and the absence of epithelium. Note the coagulated eosinophilic mucicarmine-positive material (*B*) into which round cells have migrated. Stained with hematoxylin and eosin. Orig. mag. ×290, reduced ⅛.



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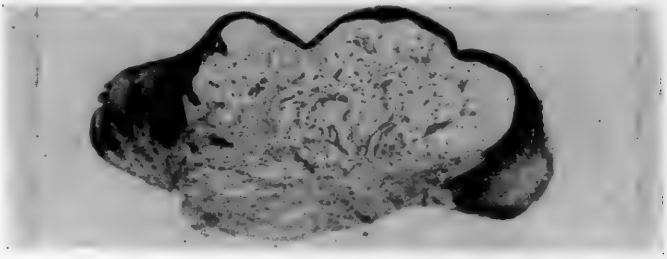

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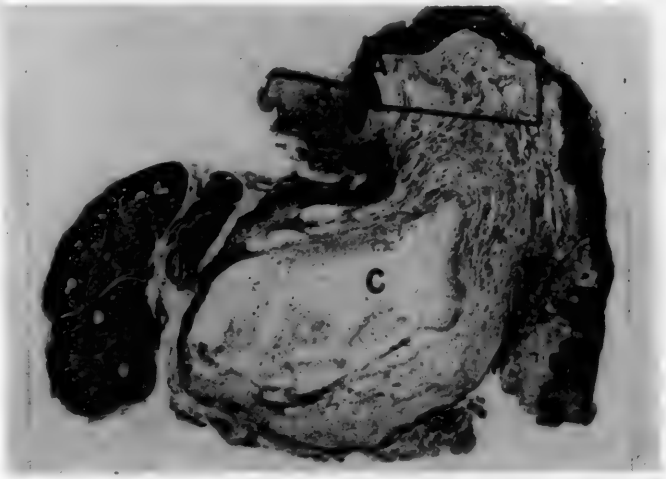


Fig. 8.

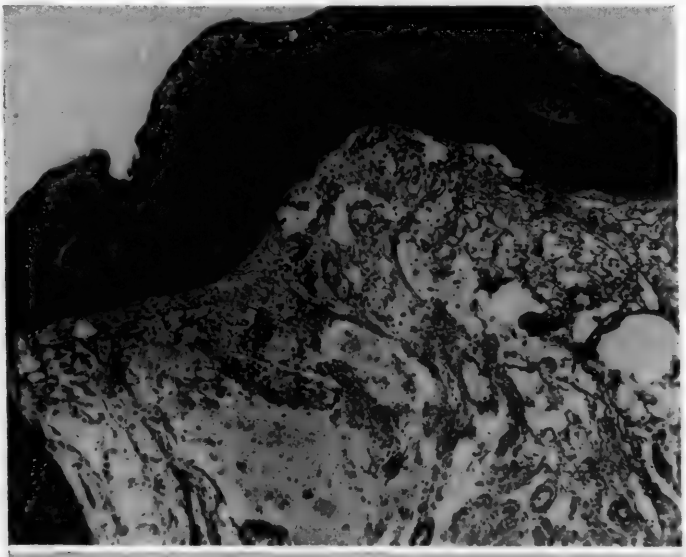
Fig. 6.—Photomicrograph of wall of mucocele (Fig. 2) showing wide layer of granulation tissue (*A*) which is edematous and infiltrated by lymphocytes and plasma cells. Note the lightly stained content of the cyst (*B*). Stained with hematoxylin and eosin. Orig. mag. ×290, reduced ⅓.

Fig. 7.—Photomicrograph of an elevated lesion on lip clinically diagnosed as mucocele. Swelling is characterized by large amounts of mucicarmine-positive material in intercellular substance of lamina propria. Note absence of epithelial ridges of the oral mucosa (*A*). The lesion represents an early mucocele, type 2. Stained with hematoxylin and eosin. Orig. mag. ×35, reduced ½.

Fig. 8.—Photomicrograph of human mucocele (type 2). Note the presence of a large cavity (*C*) and some smaller cavities (□) without definite walls in the lamina propria. Also note the lack of epithelial ridges (*A*) of the oral mucosa. Stained with hematoxylin and eosin. Orig. mag. ×20, reduced ⅙.

In one of the specimens (505-51) a type 1 mucocele showed a break in its wall. It had produced a subepithelial swelling which showed the histologic characteristics of an early mucocele of type 2 (Fig. 10). These observations suggest strongly that the cystic lesion of type 2 precedes the well-defined cystic cavity of type 1.

Fig. 9.



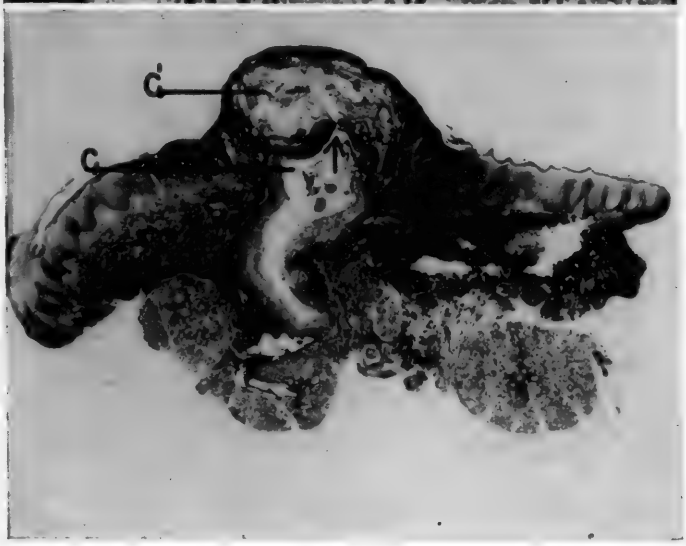


Fig. 10.

Fig. 9.—Higher magnification of an area (⊔) of Fig. 8. Note poorly circumscribed pools of mucicarmine-positive material (*B*) in lamina propria. Connective tissue separating the pools shows infiltration by neutrophils and lymphocytes. Stained with hematoxylin and eosin. Orig. mag. ×70, reduced ⅛.

Fig. 10.—Photomicrograph of a mucocele of the lower lip, type 1 (*C*) which, due to a break in wall (arrow), produced a mucocele of type 2 (*C'*). Stained with Mallory. Orig. mag. ×13, reduced ⅛.

B. *Experimental.*—Since the changes in the mice and the rats were comparable, these will be described together.

Gross findings: At sacrifice the submandibular group of glands on the experimental side appeared much larger than those on the control side.

Fig. 11. Fig. 12.

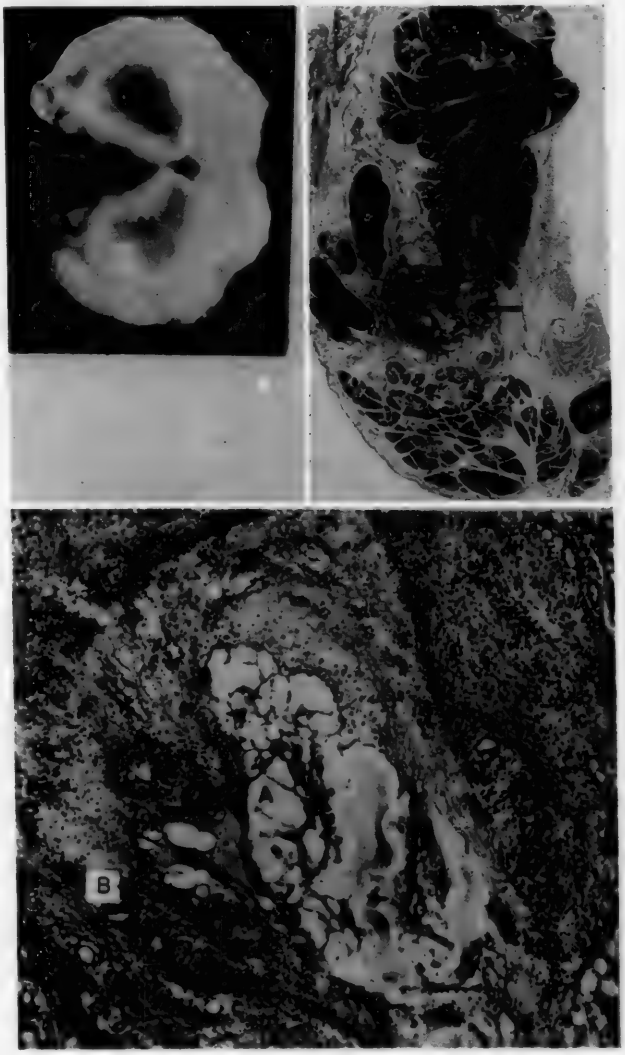


Fig. 13.

Fig. 11.—Photograph of gross specimen showing an experimental mucocele (*M*) in a rat. Excretory duct of submaxillary gland was severed 3 days previously. Orig. mag. ×2, reduced ½.

Fig. 12.—Photomicrograph of submaxillary group of glands of rat showing the accumulation of mucus in the connective tissue 1 day after severing of the excretory duct. Stained with mucicarmine. Orig. mag. ×10, reduced ½.

Fig. 13.—Higher magnification of area shown by arrow in Fig. 12. Note the mucicarmine-positive, vacuolated intercellular material (*A*) and the embedded cells (*B*). Stained with hematoxylin and eosin. Orig. mag. ×290, reduced ½.

In 5 of the 6 rats and 3 of the 5 mice the superficial surface of the glands showed either circumscribed translucent swellings or at bisection cystic cavities of varying size (Fig. 11). These cavities were filled with a clear viscous fluid which appeared like mucus. In the remaining 3 animals (1 rat and 2 mice) the submandibular area showed fistulae and the glands on bisection revealed grayish-yellow necrotic areas.

Fig. 14.

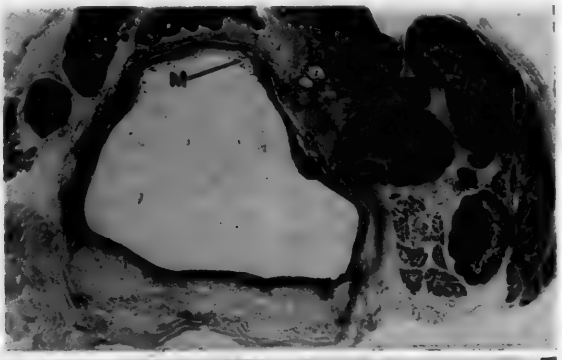
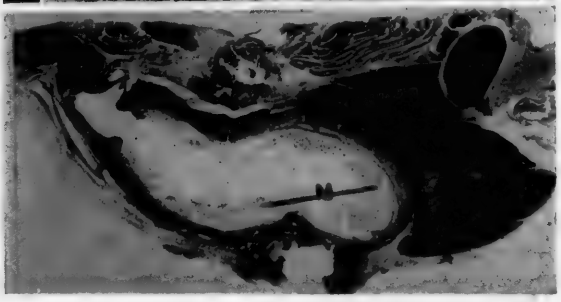


Fig. 15.

Fig. 16.



Figs. 14, 15, and 16.—Photomicrographs of mucoceles produced in the rat 2, 4, and 7 days, respectively, after severing of the excretory duct. In Fig. 16, the black line demarcates the severed dilated duct from the mucocele. Only granulation tissue lines the latter. Note mucicarmine-positive material (M) within cavities. Stained with hematoxylin and eosin. Orig. mag. ×8, ×8, ×9, respectively, reduced ⅕.

Histologic: One day after severing of the duct the connective tissue between various submandibular salivary glands and lymph nodes showed ill-defined areas of intercellular basophilic vacuolated material in which numerous cells were embedded (Figs. 12 and 13). This material was mucicarmine

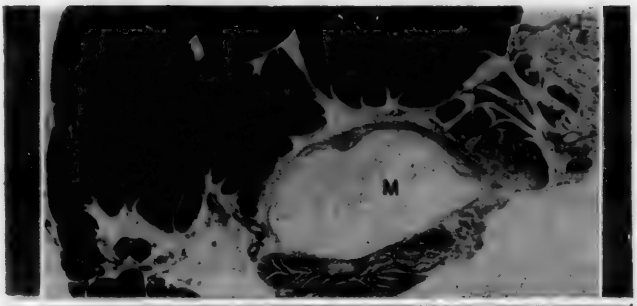


Fig. 17.

Fig. 18.

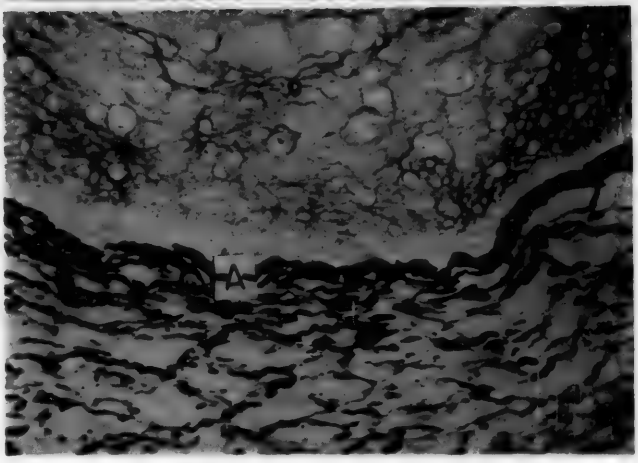


Fig. 19.

Fig. 17.—Photomicrograph of a mucocele, 3 days after severing of excretory duct in a mouse. Note mucicarmine-positive material (*M*) filling cystic cavity. Stained with hematoxylin and eosin. Orig. mag. ×12, reduced 1/11.

Fig. 18.—Higher magnification of wall of experimental mucocele shown in Fig. 16. Note absence of epithelium. Wall of cavity is formed by compressed connective tissue into which neutrophils, plasma cells, and macrophages have infiltrated (*W*). Mucicarmine-positive material containing inflammatory cells is seen within the cystic cavity (*M*). Stained with hematoxylin and eosin. Orig. mag. ×290, reduced 1/11.

Fig. 19.—Photomicrograph of wall of experimental mucocele. Higher magnification of an area of specimen shown in Fig. 14. Note the extension of the argyrophil fibers (*A*) to the surface of the cavity (*C*). Silver impregnation. Orig. mag. ×290, reduced 1/11.

positive. Although the embedded cells were all rounded their nuclei revealed that they were predominantly neutrophilic leukocytes but some macrophages and fibroblasts were also present. The connective tissue surrounding and separating the mucous pools showed varying degrees of neutrophil infiltration.

From 2 to 9 days after the severing of the duct typical mucoceles developed which were comparable to human lesions (Figs. 14 to 17). These cystic cavities varied in size from about 3 by 3 by 3 mm. to 12 by 12 by 9 mm. These cavities contained mucicarmine-positive material. In cases where the cysts were bisected prior to embedding, the mucicarmine-positive material had escaped and only small amounts of it could be seen along the wall of the cavities. Embedded within the mucicarmine-positive material were numerous rounded cells, the nuclei of which resembled those of neutrophils, macrophages, or fibroblasts (Fig. 18). With increasing postoperative period the number of neutrophils diminished. The walls of the cysts were not lined by epithelium and in sections stained with silver, argyrophil fibers extended to the cystic cavity (Fig. 19). In early stages the cystic wall consisted predominantly of preexisting compressed connective tissue, but in later stages fibroblastic proliferation had occurred. The cavity was then lined by a wide band of granulation tissue. The inner surface of the cyst wall showed transitions between normal young fibroblasts and the balloon-like cells in the cystic fluid. The connective tissue adjacent to the wall of the cystic lesions showed varying degrees of infiltration by neutrophil and plasma cells and an abundance of macrophages.

In one of the specimens (7 days) the section happened to pass through the severed salivary duct. This specimen showed the cut end of the duct opening into the cystic cavity. The epithelial lining of the dilated duct, however, ended abruptly and did not extend over the cystic wall (Fig. 16).

The submaxillary and sublingual glands on the experimental side showed varying degrees of interstitial infiltration by lymphocytes and plasma cells and atrophy of the acinar and tubular cells.

In 3 animals in which mucoceles did not develop, circumscribed areas of coagulation necrosis involving both the sublingual and submaxillary gland were present. All 3 specimens showed numerous small, deeply basophilic foci of bacterial colonies. In one case thrombosis of a large artery and calcification of the media could be observed.

DISCUSSION

1. *Lining of Mucoceles.*—It is generally considered that the mucoceles and ranulae are lined by epithelium. In our material, however, epithelial lining could not be demonstrated in any of the cysts. Since in hematoxylin and eosin-stained specimens it was sometimes difficult to ascertain whether the lining cells were fibroblasts or flattened epithelial cells, sections were stained with silver. These revealed that the argyrophilic fibers in varying density extended to the very surface of the cavity. Although in our material mucoceles do not show epithelial lining, it is theoretically possible that in some

cases such a lining may develop secondarily. In one of our specimens (Fig. 16) the experimentally severed duct opened directly into a cystic cavity but the epithelial lining was restricted to the duct. It could be assumed that in such a case at a later stage the epithelium of the duct would proliferate and line the cystic space. The experimental as well as the human material in the present investigation has shown, however, that generally the mucoceles are lined in early stages by compressed pre-existing connective tissue and in later stages by granulation tissue only.

2. *Pathogenesis of Mucoceles.*—Mucoceles are believed to be retention cysts.[1] However, in experimental obstruction (ligation) of the ducts of submaxillary and sublingual glands in mice these lesions could not be produced.[2] These findings showed that obstruction per se was probably not the cause of mucoceles. Furthermore, the study of human lesions showed that early stages of clinical mucoceles were characterized by accumulation of mucus in the lamina propria and submucosa (Figs. 7 to 9). These accumulations of mucus did not have clear-cut boundaries. If these lesions were due to duct obstruction one would expect not only clearly demarcated but epithelium-lined pools of mucus.

In the present investigation, when the salivary ducts were severed in mice and rats, lesions identical to human mucoceles developed. In early stages there was an accumulation of mucus in the connective tissue, but with continuous pooling of saliva a clearly demarcated cavity developed. This was lined by compressed connective tissue and later by granulation tissue. Similar developmental stages have been demonstrated in the biopsy material. These findings along with the fact that mucoceles are most frequently found in areas most exposed to trauma, i.e., the lower lip, indicate that a cut or traumatic defect of a salivary duct produces mucoceles.

SUMMARY AND CONCLUSIONS

This investigation of the pathogenesis of mucoceles consists of two parts. In Part I, which is based on a study of 19 surgically removed specimens, the morphology of the human mucoceles is described. Part II consists of an experimental production of mucoceles by severing the salivary ducts in 5 mice and 6 rats. All specimens, human as well as experimental, were fixed in 10 per cent formalin, embedded in paraffin, sectioned serially, and stained with hematoxylin and eosin, silver impregnation, Mallory's connective tissue stain, Mayer's Mucicarmine, and Van Gieson's method. The findings and conclusions were:

1. Mucoceles are not lined by epithelium but by connective tissue or granulation tissue.

2. In early stages mucoceles show an ill-defined accumulation of mucus in the lamina propria and submucosa, but as the salivary secretion continues well-defined cystic cavities containing mucin-positive material develop.

3. In 8 of the 11 experimental animals lesions similar to human mucoceles could be reproduced. The 3 animals in which mucoceles did not form showed secondary infection and extensive necrosis.

4. Mucoceles in man are apparently produced by a traumatic defect or severing of a salivary duct and the subsequent escape of saliva into the adjacent tissues.

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OVARIAN FUNCTION IN PERIODONTOSIS

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PERIODONTOSIS occurs predominately in females of reproductive age, particularly during adolescence and early maturity. Because of this fact, it was thought that a study of ovarian function in female patients with periodontosis might yield an etiological clue. Also, since there is considerable destruction of alveolar bone in periodontosis, and since the generalized osteoporosis sometimes exhibited by postmenopausal women is improved by sex hormone administration,[1, 2] we felt that there was further reason for a study of sex hormone production in our patients. A previous report[3] was concerned with the elaboration of urinary neutral 17-ketosteroids, as an index of androgen production, in 33 female patients with periodontosis. The distribution of values was within the normal range, with a mean for the group that did not differ significantly from that of a group of females of comparable age who were free from periodontal disease. In the present study we sought to delineate the adequacy of female hormone production by a study of serial vaginal smears and the basal body temperature recordings during the menstrual cycle.

While the menstrual cycle generally is 28 days in length, normally, it may vary considerably. The cycle is divisible into two phases. The follicular phase, from the initiation of menstrual flow to the time of ovulation, is characterized by increasing production of estrogenic hormone by the ripening ovarian follicle. The amount of estrogen produced during this period may be estimated by measurement of urinary estrogen excretion, or may be demonstrated biologically by the use of the vaginal smear. The vaginal mucosa is very sensitive to estrogen stimulation, and exfoliated cells, aspirated to make a smear, will indicate, when appropriately stained, the extent of estrogen production. Normally, during the follicular phase, successive daily vaginal smears stained by a trichrome method will demonstrate a progressively increasing number of red-staining cornified cells, reflecting a rise in estrogen production. With ovulation cornification reaches a peak, and there is a slight fall in the basal body temperature. Shortly thereafter, the vaginal smear shows a pronounced drop in cornification, and the presence of considerable amounts of mucus, cellular debris, and leukocytes. The ruptured follicle is soon reorganized into the corpus luteum, which produces the second ovarian hormone, pro-

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gesterone. The *luteal phase,* between ovulation and the beginning of the next menstrual period, is characterized by the hormonal product of the corpus luteum. The vaginal smear during this phase shows predominately the effects of progesterone, with the appearance of characteristically clumped and folded blue-staining cells, and considerable amounts of mucus. Another notable event during the luteal phase is the sustained rise of basal body temperature attributable to the action of progesterone. Hormonal deficiencies, and the presence or absence of ovulation, may be ascertained by a cytological study of the serial smears, in conjunction with the basal body temperature recording.

METHODS

The indices of ovarian activity employed in this study include: (a) serial vaginal smears, taken every other day, throughout at least one complete cycle; (b) daily basal body temperatures, taken immediately upon arising throughout the cycle; and (c) in some patients serial *oral* smears, as carried out by Ziskin and Moulton.[4] The oral smears were taken from the gingivae on the left side of the mouth by lightly passing a thin flat blade (spatula) over the tissue, simultaneously with the taking of the vaginal smears. Ziskin and Moulton had reported that the major changes in cornification of the vaginal smear were reflected in the oral smear. The smears were stained by the trichrome method developed by Shorr.[5] The vaginal smears were studied for evidence of cyclic change, presence or absence of ovulation, and quality of hormone production. The oral smears were compared with the vaginal smears for demonstration of cycle quality and as an index of ovulation, and also for the possibility of delineating a distinctive oral smear picture in periodontosis.

RESULTS

Of the 33 patients studied by vaginal smears and temperature records (Table I), all but 3 had ovulatory cycles. Of the ovulatory series, on the basis of the smear evidence, 19 were considered to be of good quality, 8 were judged to be of fair quality, and 3 of poor quality. The 3 patients who failed to ovulate showed evidence of moderate to marked estrogen insufficiency. Thus a considerable range of ovarian activity was demonstrable for the group, with the majority showing adequacy of the cycle.

It is of interest to compare the severity of periodontal disease with the quality of the ovarian cycle. The system of grading the degree of periodontal involvement is as follows: slight: bone loss in specific areas without the necessity of immediate extraction of teeth; moderate: bone loss to an extent requiring extraction of some teeth, which can be replaced by prosthesis; severe: more extensive bone loss requiring extraction of all but 6 teeth. That there is a suggestion of correlation between the quality of ovarian activity and the degree of periodontal involvement is shown by the predominance of slight and moderate cases in the group with evidence of a qualitatively good ovarian cycle. There were 15 such cases in a group of 19. In contrast to this, there were only 6 slight and moderate cases in the combined group of 14 showing fair, poor, and anovulatory cycles. Stating it somewhat differently, there were

TABLE I
PERIODONTOSIS AND OVARIAN FUNCTION

AGE	QUALITY OF CYCLE	DEGREE OF PERIODONTOSIS	AGE	QUALITY OF CYCLE	DEGREE OF PERIODONTOSIS
30	Good	Slight	31	Fair	Slight
34	Good	Slight	25	Fair	Moderate
31	Good	Slight	19	Fair	Moderate
16	Good	Slight	30	Fair	Moderate
29	Good	Slight	34	Fair	Severe
14	Good	Slight	34	Fair	Severe
30	Good	Slight	31	Fair	Severe
33	Good	Slight	17	Fair	Severe
42	Good	Moderate	13	Poor	Slight
19	Good	Moderate	22	Poor	Moderate
43	Good	Moderate	36	Poor	Severe
37	Good	Moderate	25	Anovulatory	Severe
31	Good	Moderate	20	Anovulatory	Severe
29	Good	Moderate	22	Anovulatory	Severe
30	Good	Moderate			
28	Good	Severe			
39	Good	Severe			
27	Good	Severe			
34	Good	Severe			

only 4 severe cases in the group of 19 showing good ovarian cycles against 8 severe cases in the group of 14 showing fair, poor, and anovulatory cycles. It should be noted that there was severe involvement in all 3 subjects with anovulatory, estrogen-deficient cycles. Age, per se, does not seem to be a determining factor in the degree of periodontal involvement since the average age in all 3 groups was about the same.

In 16 cases, simultaneously obtained vaginal and oral smears were studied. Parallelism was striking with point-for-point correspondence throughout the cycle in 8 cases, covering a wide range of ovarian function. One of these patients exhibited an anovulatory, estrogen-deficient vaginal series, with oral smears demonstrating low cornification throughout. Three patients showed essential cyclic correspondence in both series, but indication of ovulation later by 2 to 3 days in the oral series, as compared to the vaginal smears and temperature records. For 5 subjects there was no correspondence between the vaginal and oral series, 1 patient showing an anovulatory estrogen-deficient pattern of vaginal smears, no indication of ovulation from the temperature record, but uniformly high cornification on the oral smears, though without evidence of cyclic activity. There were 2 patients with moderate to severe periodontal involvement, and indication of ovulation by the vaginal series which demonstrated fair to good cycle quality, in whom cyclic changes in the oral smears were possibly obscured by local changes in the gingival mucosa. The oral smears showed persistent "sheeting" or exfoliation of layers of uniform, large-nucleated blue-staining cells. One patient showing essentially good correspondence between the vaginal and oral series, with a cycle of good quality and with only slight periodontal disease, also demonstrated some intermittent sheeting in the oral series.

Of interest in the good correspondence group was the parallelism, not only in cornification during the follicular phase, but also the appearance on the oral

smears of the clumped, folded cells characteristic of the vaginal luteal phase. It is probable that there is a gradation of epithelial tissue sensitivity to hormone effect ranging from the most sensitive (vaginal mucosa) through other mucous membranes, such as the oral, and extending to surface epithelium, which undergoes involutional change with estrogen insufficiency after the menopause, with restoration to normal after the administration of estrogen.[6]

It is of interest that even subjects with considerable gingival involvement secondary to the disease process in the alveolar bone can still demonstrate on oral smears cyclic changes dependent on the changing endocrine environment during the menstrual cycle.

SUMMARY

1. Thirty-three female patients with periodontosis were studied to ascertain the quality of ovarian activity, employing indices of: serial vaginal smears, simultaneous oral smears (in 16 cases), and basal body temperature records.

2. Of the 33 subjects studied by vaginal smears, alone or in conjunction with oral smears and by temperature records, 19 showed ovulatory cycles of good quality, 8 of fair quality, and 3 of poor quality. Three patients with anovulatory cycles showed evidence of moderate to marked estrogen deficiency.

3. There was some correspondence between the severity of periodontal disease and the quality of the ovarian cycle, with 15 of the 19 subjects demonstrating ovarian cycles of good quality showing only slight or moderate periodontal involvement, in contrast to 6 of the 14 subjects demonstrating fair, poor, and anovulatory cycles showing only slight or moderate periodontal involvement. All 3 anovulatory cases showed severe involvement.

4. Of the 16 patients in whom simultaneously obtained vaginal and oral smears were studied, 11 showed good correspondence between the series, although in 3 of these there was an indication of ovulation later by 2 to 3 days in the oral series. Five patients showed no correspondence. Two patients with moderate to severe periodontal disease showed fair to good vaginal cycles, but acyclic oral smears with persistence of exfoliation of sheets of cells in the oral series. Intermittent "sheeting" was also observed in a corresponding oral series of a patient with a vaginal cycle of good quality, and slight periodontal involvement.

5. There was no distinctive oral smear picture of periodontosis, though the observed "sheeting" may be related to local gingival involvement.

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A LONGITUDINAL STUDY OF DENTAL ARCH WIDTH AT THE DECIDUOUS SECOND MOLARS ON CHILDREN 4 TO 8 YEARS OF AGE

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PREVIOUS research "concerning the natural growth of the width . . . of the dental arches"[1] has been reported by Allen,[2] Alley,[3] Brawley and Sedwick,[4] Channing and Wissler,[5] Cohen,[6] Goldstein and Stanton,[7, 8] Hellman,[9] Henriques,[10] Lancet,[1] Lewis and Lehman,[11] Meredith and Cox,[12] Sillman,[13] Smyth and Young,[14] Wallace,[15] and Woods.[16] Of the North American investigations, only those by Cohen[6] and Woods[16] "follow throughout a long span of time the process of growth in each child of a representative group of children."[7] Cohen worked from dental casts, Woods from roentgenograms; each used a sample of less than thirty children; neither dealt with width between the buccal surfaces of the deciduous second molars.

OBJECTIVES

Major aims of the present investigation are:

1. To present findings on the reliability of records for maximum rectilinear distance between the buccal surfaces of the deciduous second molars. Reliability coefficients are reported for each dental arch at 3 different ages.

2. To statistically describe interbuccal width at the deciduous second molars on 12 age-arch-sex subgroups of North American white children. Central tendency and variability values are tabled, specific for dental arch and sex, at ages 4, 6, and 8 years.

3. To analyze longitudinal data pertaining to growth in dental arch width between 4 and 8 years of age. For a series of 77 children, changes with age in transverse diameter between the lateral-most points of the deciduous second molars are subgrouped according to arch and sex, and the resulting distributions portrayed and discussed.

4. To investigate a number of dental arch relationships. Correlation statistics are used to depict the association between (a) arch width at 4 years and arch width at 8 years, (b) arch width at 4 years and change in arch width from 4 to 8 years, (c) maxillary width and mandibular width at 4 years, (d) change in maxillary and mandibular widths from 4 to 8 years, (e) maxillary width and upper face width at 6 years, and (f) mandibular width and lower face width at 6 years.

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SUBJECTS

The subjects were 40 boys and 37 girls living in or near Iowa City, Iowa, and enrolled in a long-term research program begun at the State University of Iowa in 1946 under the joint sponsorship of the Department of Orthodontics and the Iowa Child Welfare Research Station.

At the time of enrollment in the program there were 4 criteria for acceptance: age (under 4 years), race (white), geographic location (vicinity of Iowa City), and availability for longitudinal study (willingness to participate, and likelihood of continuing residence in the region). Five additional criteria were applied in selecting subjects for the present study: each child was required to have (a) maxillary and mandibular dental casts on file at 4, 6, and 8 years of age; (b) 4 erupted deciduous second molars present at each age; (c) 4 erupted deciduous first molars present at each age; (d) all buccal surfaces of the deciduous second molars free of restorative dental work; and (e) a developmental record showing no orthodontic treatment.

The children were American-born and mainly of northwest European ancestry. For 75 per cent, all 4 grandparents were immigrants or descendants of immigrants from the British Isles, Germany, Scandinavia, France, or the Netherlands; 22 per cent had either (a) 3 grandparents of northwest European lineage and 1 of central European lineage, or (b) 2 grandparents of northwest European lineage and 2 of eastern, central, or southern European lineage, while the remaining 3 per cent comprised one subject of each sex whose progenitors came from eastern and central Europe.

With reference to socioeconomic status, the subjects are predominantly from the professional, managerial, and commercial groups. Analysis of data on occupation of the fathers yields the following: in the professional and managerial categories, 68 per cent; in the commercial and skilled trades categories, 23 per cent; and in the unskilled category, 9 per cent.

PROCEDURES IN OBTAINING DATA

The source materials were dental stone casts made from alginate impressions. Special care was used in taking the impressions and in preparing the casts. In practically all instances the impressions were obtained on the child's fourth, sixth, and eighth birthdays, or within 2 weeks of these dates.

On each maxillary and mandibular dental cast, the maximum rectilinear distance was determined between the buccal surfaces of the right and left deciduous second molars. All measuring was done with sliding vernier calipers checked for accuracy against a standard millimeter scale. The arms of the calipers were first brought in contact with the most distal (posterior) regions of the buccal surfaces and then applied in successively more medial regions until the greatest transverse diameter was found. Measurements were made to the nearest one-tenth millimeter, independently, by both investigators.

RELIABILITY OF MEASUREMENTS

Applying the Pearson product-moment method of correlation (r) to the series of paired records for each age, sex, and dental arch, the coefficients in Table I were obtained.

In addition, the difference between each pair of records was derived. Every record obtained by the one measurer was subtracted from the corresponding record of the other measurer, and the entire series of differences tabulated in one distribution. This was statistically defensible since the separate distributions from subgrouping by sex, age, and dental arch were equivalent. It follows that the 40 differences for males at each of the ages 4, 6, and 8 years on the maxillary and mandibular arches, plus the 37 differences for females at each of these ages on both arches, gave a total of 462 values for tabulation. Findings from the distribution are: (1) its standard deviation is 0.12 mm., and (2) 96 per cent of the differences lie between zero and 0.2 mm.

TABLE I
RELIABILITY COEFFICIENTS FOR MAXIMUM TRANSVERSE DIAMETER BETWEEN THE BUCCAL SURFACES OF THE DECIDUOUS SECOND MOLARS MEASURED ON DENTAL CASTS OF CHILDREN 4, 6, AND 8 YEARS OF AGE

	MAXILLARY ARCH				MANDIBULAR ARCH			
	MALES		FEMALES		MALES		FEMALES	
AGE	N	r	N	r	N	r	N	r
4	40	0.994	37	0.995	40	0.995	37	0.993
6	40	0.992	37	0.992	40	0.995	37	0.993
8	40	0.996	37	0.998	40	0.997	37	0.998

N = number of subjects.
r = Pearson product-moment correlation coefficient.

An earlier study[12] had found no difference between arch width per se (determined from series of measurements taken directly in the mouth) and arch width obtained via cast replication and repeated measurement.

Summarizing for reliability and validity, the data on arch width utilized in this study are highly dependable. Both systematic and chance error are adequately minimized.

ARCH WIDTH AT 4, 6, AND 8 YEARS OF AGE

The measurements of dental arch width used from this juncture forward are composite values. In most instances they are values derived by averaging 2 independent records for a given arch. For the small percentage of cases where the 2 independent records differed by more than 0.2 mm., each measurer made a second measurement, and the composite values are averages of 4 determinations.

Central Tendency.—The series of values for each of the 12 arch-sex-age subgroups were analyzed separately. Columns 3 and 7 of Table II display the means obtained. It will be seen:

1. Maximum transverse diameter between the buccal surfaces of the deciduous second molars increases with age. Generalizing for the 2 arches and both sexes, there is an average increase of 0.8 mm. between ages 4 and 6 years, and 0.9 mm. between ages 6 and 8 years.

2. The mandibular arch is narrower than the maxillary arch. Generalizing for both sexes and all 3 ages, the means for the maxillary arch are larger than those for the mandibular arch by 3.1 mm.

3. Both of the dental arches are wider in males than in females. Generalizing for the 3 ages and 2 arches, there is an average sex difference of 1.9 mm.

The over-all differences obtained for sex (1.9 mm.) and age (1.7 mm.) suggest that interbuccal width of the arches at the deciduous second molars is no greater in the average female at age 8 years than in the average male at age 4 years. Table II confirms this.

TABLE II
MEASURES OF CENTRAL TENDENCY (MM.) AND VARIABILITY FOR MAXIMUM INTERBUCCAL WIDTH OF THE DENTAL ARCHES AT THE DECIDUOUS SECOND MOLARS ON NORTH AMERICAN WHITE CHILDREN 4, 6, AND 8 YEARS OF AGE

AGE	MALES				FEMALES			
	N	MEAN	S.D.	V	N	MEAN	S.D.	V
			Maxillary arch					
4	40	47.7*	2.3	4.7	37	46.1	2.2	4.8
6	40	48.8	2.5	5.2	37	47.0	2.1	4.5
8	40	49.8	2.5	5.1	37	47.7	2.3	4.9
			Mandibular arch					
4	40	45.0	1.7	3.8	37	43.1	1.9	4.5
6	40	45.7	1.9	4.0	37	43.7	1.9	4.5
8	40	46.6	1.8	3.9	37	44.5	2.0	4.6

S.D. = standard deviation (mm.)
V = coefficient of variation (%).
*The standard errors are not listed individually. Their magnitude varies from 0.3 mm. to 0.4 mm. for the means, and from 0.2 mm. to 0.3 mm. for the standard deviations.

Additional central tendency findings accrue from comparison of the Table II means with means from previous studies. Goldstein and Stanton[8] measured "the greatest distance between the right and left . . . buccal aspects of the deciduous second molars," in both arches, on 60 American-born, white males 5 and 8 years of age selected as having "normal dental occlusion." Their means are no larger (explicitly, they average 0.4 mm. smaller) than those of the present study.

Another study by Goldstein and Stanton[7] utilized 300 children between 4 and 8 years of age "with both normal and abnormal occlusion." These children were of both sexes, "mainly of the middle class . . . preponderantly of American stock," and untreated orthodontically. In each dental arch, the means for "maximum width between buccal aspects, at gum line, of deciduous second molars" are, on the females, practically the same as those of the present study and, on the males, around 0.8 mm. lower than those of the present study.

Alley,[3] Cohen,[6] and Lewis and Lehman[11] studied transverse distances between the deciduous second molars using termini lingual to the buccal surfaces. Their subjects were drawn in New York, Minneapolis, and Detroit.

On the maxillary arch, the distance between "the points of the mesio-lingual cusps" was measured by Cohen, and by Lewis and Lehman. Alley (a) reproduced "the bucco-lingual outline of the form of the teeth at the gingival margin, together with . . . the mesio-distal-contact diameters of the teeth," and (b) located each terminus as "the point of intersection of the central mesio-distal and bucco-lingual diameters." The maxillary means of

the present study exceed Alley's bicentroid means by 9.8 mm. They are larger than the two series of bimesiolingual-cusp means by 13.4 mm. (Cohen) and 13.6 mm. (Lewis and Lehman).

The width termini were the same for the mandibular arch as for the maxillary arch in the Alley and Cohen studies. Lewis and Lehman measured the distance between "the central fossae" of the lower deciduous second molars. In comparison with the mandibular means of the present study, the bicentroid means of Alley are smaller by 8.7 mm., the bicentral-fossa means of Lewis and Lehman smaller by 10.3 mm., and the bimesiolingual-cusp means of Cohen smaller by 15.1 mm.

Variability.—The spread of the individual measurements within each arch-age-sex subgroup is described in 2 ways: (1) by the standard deviation and coefficient of variation, and (2) by a selected group of percentiles. The standard deviation summarizes the dispersion of a distribution in one value, thereby facilitating comparison of the over-all variability of different distributions. Selected percentiles characterize a distribution less succinctly, but provide a conveniently usable description of individual differences for clinical reference.

Tables II and III present the variability results. Selected findings from Table II are:

1. The standard deviations for the 12 arch-age-sex subgroups vary between 1.7 mm. and 2.5 mm. Corresponding values for the coefficient of variation fall between 3.8 per cent and 5.2 per cent.

2. For males, maxillary arch width is more variable than mandibular arch width. This is true both absolutely, as expressed by the standard deviations, and relatively, as expressed by the coefficients of variation. A like finding was secured in an earlier study of "maximum transverse diameter between the permanent first molars" on 9-year-old males.[12] Here, the specific S.D. and V values were 2.6 mm. and 4.6 per cent for the maxillary arch, 2.0 mm. and 3.7 per cent for the mandibular arch.

3. The indication of greater variability in maxillary arch width for females (as well as males) harmonizes with the standard deviations from sex-arch subgrouping obtained by Cohen.[6] Further, the standard deviations reported by Alley,[3] Goldstein and Stanton,[7] and Lewis and Lehman,[11] for both sexes combined, are slightly higher on the maxillary arch than on the mandibular arch.

Table III shows:

1. At 4 years of age, 10 per cent of the maxillary arch width measurements for males were less than 44.2 mm., 50 per cent between 46.2 mm. and 49.3 mm., and 10 per cent more than 50.6 mm. Correspondingly for 4-year-old females, the narrowest one-tenth of the maxillary arch widths were below 43.4 mm., the middle one-half between 44.9 mm. and 47.4 mm., and the widest one-tenth above 48.9 mm.

2. For 80 per cent of the males studied at age 6 years, mandibular arch width was between 42.6 mm. (tenth percentile) and 48.0 mm. (ninetieth percentile). Eighty per cent of the 6-year-old females had mandibular widths between 41.2 mm. and 46.1 mm.

3. Two 8-year-old children, 1 male and 1 female, had maxillary arch widths of 47.0 mm. Reference to Table III shows the arch of the male to be moderately narrow and that of the female average. Two other children the same age, one of each sex, had mandibular arch widths of 47.0 mm. Table III indicates that the arch of the male is about average and that of the female moderately wide. These examples suffice to illustrate the clinical usefulness of Table III as a frame of reference.

TABLE III
VALUES (MM.) REPRESENTING TWELVE DISTRIBUTIONS FOR MAXIMUM INTERBUCCAL WIDTH OF THE DENTAL ARCHES AT THE DECIDUOUS SECOND MOLARS ON NORTH AMERICAN WHITE CHILDREN 4, 6, AND 8 YEARS OF AGE

AGE	SEX	MINIMUM	PERCENTILES				MAXIMUM
			10	25	75	90	
			Maxillary arch				
4	Males	41.9	44.2	46.2	49.3	50.6	51.7
	Females	38.8	43.4	44.9	47.4	48.9	50.6
6	Males	42.8	45.1	47.0	50.6	52.1	52.9
	Females	40.1	44.6	45.7	47.9	49.6	51.5
8	Males	44.3	46.0	48.0	51.8	53.0	54.2
	Females	40.6	45.2	46.1	48.6	51.5	52.5
			Mandibular arch				
4	Males	41.2	42.2	43.8	46.2	47.0	48.1
	Females	38.6	40.2	41.7	44.4	45.6	46.7
6	Males	41.2	42.6	44.5	47.1	48.0	48.4
	Females	39.1	41.2	42.5	44.9	46.1	48.3
8	Males	42.6	43.4	45.5	48.0	48.9	49.7
	Females	40.3	41.4	43.3	45.8	47.3	48.8

CHANGE IN ARCH WIDTH BETWEEN 4 AND 8 YEARS OF AGE

Since measurements were obtained on every subject at 4 years of age and exactly 4 years later, it was possible to analyze the changes in arch width over this 4-year span. For each of the 77 subjects, maxillary width at 4 years was subtracted from maxillary width at 8 years. The same procedure was followed for the mandibular arch. Tables IV and V display central tendency and variability statistics from these data.

TABLE IV
MEANS AND STANDARD DEVIATIONS (MM.) FOR INCREASE BETWEEN 4 AND 8 YEARS OF AGE IN MAXIMUM INTERBUCCAL WIDTH OF THE DENTAL ARCHES AT THE DECIDUOUS SECOND MOLARS

DENTAL ARCH	MALES			FEMALES		
	N	MEAN	S.D.	N	MEAN	S.D.
Maxillary	40	2.1	0.80	37	1.6	1.02
Mandibular	40	1.6	0.82	37	1.4	0.82

Central Tendency.—Findings with reference to Table IV are:

1. Taking the 4 arch-sex subgroups together, the mean increase between 4 and 8 years of age in maximum interbuccal width at the deciduous second molars is 1.7 mm. Expressed in annual terms this represents an average gain of approximately 0.4 mm. per year.

2. Combining the sexes, the mean increment between 4 and 8 years of age is 1.9 mm. in the maxillary arch, and 1.5 mm. in the mandibular arch. Cohen,[6] from his study of width between the mesiolingual cusps on 28 children of both sexes, obtained means for the same 4-year period of 1.1 mm. on each arch. It is possible that the higher increment means of the present investigation are due, at least partly, to control on the variable of forward migration of deciduous second molars, one requirement in sample selection being presence of all 4 deciduous first molars at every age.

3. For the maxillary dental arch, the mean increase between 4 and 8 years of age is 2.1 mm. for males, and 1.6 mm. for females. This difference between the sexes is statistically significant at the 2 per cent level of confidence ($t = 2.46$). For the mandibular arch, the mean increase from 4 to 8 years of age is 1.6 mm. for males, and 1.4 mm. for females. Here, there is not a statistically significant sex difference.

TABLE V
VALUES (MM.) DEPICTING FOUR DISTRIBUTIONS FOR INCREASE IN MAXIMUM INTERBUCCAL WIDTH OF THE DENTAL ARCHES AT THE DECIDUOUS SECOND MOLARS OVER THE PERIOD FROM 4 TO 8 YEARS OF AGE

SEX	MINIMUM	PERCENTILES				MAXIMUM
		10	25	75	90	
			Maxillary arch			
Males	0.0	0.8	1.7	2.6	2.9	3.4
Females	−0.5	0.4	0.8	2.2	3.3	3.8
			Mandibular arch			
Males	0.0	0.6	1.0	2.0	2.6	3.5
Females	−0.5	0.5	0.9	1.9	2.4	3.6

Variability.—Table V yields findings of the following order:

1. There are individuals of both sexes who show no increase whatever in dental arch width between 4 and 8 years of age. At the other extreme, there are individuals of both sexes who register orthodontically uninfluenced width increases in the vicinity of 3.5 mm.

2. For the maxillary arch, 80 per cent of the males increased between 0.8 mm. and 2.9 mm. over the 4-year period. On the mandibular arch, 10 per cent of the increases are less than 0.6 mm., 50 per cent between 1.0 mm. and 2.0 mm., and 10 per cent more than 2.6 mm.

Additional material pertaining to normal growth in dental arch width at the deciduous second molars is available from Ainsworth,[17] Chapman,[18, 19] Goldstein and Stanton,[8] Lancet,[1] Schwarz,[20, 21] Stanton,[22] and Wallace.[15] Goldstein and Stanton report group analyses covering annual intervals, the others give increments for individuals over periods varying from 2 years to 6 years.

Table VI brings together the increments for individuals. Ainsworth, Chapman, Lancet, and Schwarz took interbuccal measurements, Stanton bicentroid measurements, and Wallace interlingual measurements. All of the children were described as presenting normal occlusion,[18, 19, 22] normal dental arches,[15, 20] or developmental conditions uninfluenced by orthodontic treatment.[1, 17, 21]

Goldstein and Stanton analyzed data for change in width between the buccal aspects of the deciduous second molars obtained on groups of children measured at 2 or more ages 1 year apart. Table VII reproduces their findings for 3 annual periods on children with normal occlusion.

TABLE VI
INCREMENTS FOR WIDTH AT THE DECIDUOUS SECOND MOLARS ON INDIVIDUAL CHILDREN WITH NORMAL DENTAL ARCHES

INVESTIGATOR	AGE INTERVAL	INCREASE IN MILLIMETERS	
		MAXILLARY ARCH	MANDIBULAR ARCH
Chapman (1915)	4.0- 9.2*	2.2	1.8
Schwarz (1925)	3.5-10.0	3.0	
Lancet (1930)	5.5- 7.5	1.1	1.2
Schwarz (1932):	4.0- 7.0	2.0	
	4.0- 7.0	1.5	
	5.7-10.0	1.0	
Ainsworth (1935)	6.5-10.0*	2.7	1.8
Chapman (1935):	3.8- 6.0**	1.0	
	5.6- 9.4**	3.5	
Stanton (1935)	4.8- 6.9	1.5	0.9
Wallace (1937)	3.5- 8.2*	2.4	0.6

*Males.
**Females (sisters).

TABLE VII
FINDINGS FROM GOLDSTEIN AND STANTON FOR ANNUAL CHANGE IN ARCH WIDTH AT THE DECIDUOUS SECOND MOLARS ON WHITE AMERICAN-BORN CHILDREN OF BOTH SEXES HAVING NORMAL OCCLUSION

AGE INTERVAL	N	PERCENTAGE OF CHILDREN SHOWING CHANGE OF:		
		LESS THAN −0.2 MM.	−0.2 MM. TO +0.2 MM.	MORE THAN +0.2 MM.
		Maxillary arch		
3-4	29	17	28	55
5-6	61	18	30	52
7-8	31	3	23	74
		Mandibular arch		
3-4	36	28	30	42
5-6	59	12	35	53
7-8	28	4	32	64

CORRELATION FINDINGS

Relations Between the Two Arches.—The relationship of the maxillary arch to the mandibular arch was studied in 2 ways: (1) by correlating widths of the 2 arches at 4 years of age, and (2) by correlating width changes in the 2 arches between 4 and 8 years of age. For both purposes, the Pearson product-moment method of expressing concomitance was employed.

For maxillary interbuccal width between the deciduous second molars at 4 years, with the corresponding mandibular arch diameter at this age, the

obtained coefficients of correlation are 0.73 on the 40 males, and 0.89 on the 37 females. These *r*'s are of the same order as those secured earlier (0.76 and 0.79) for interbuccal width at the permanent first molars on children 9 years of age.[12]

Investigation of the association between change in maxillary interbuccal width at the deciduous second molars over the age period 4 to 8 years, and change in the corresponding mandibular dimension over the same period, yields *r*'s of 0.67 and 0.77 on males and females, respectively. Summarizing with reference to both width and change in width, the 2 arches are moderately, but not highly, related.

It follows that predictions of size or change in one arch from information regarding size or change in the other would be subject to considerable error. In statistical terms, the indices of prediction efficiency for *r*'s of the above magnitude lie between 25 and 55 per cent.[23]

Relations Pertaining to Age.—For each arch separately, 2 problems were studied: (1) the relationship between arch width at age 4 years and arch width at age 8 years, and (2) relationship between arch width at age 4 years and change in arch width over the age period 4 to 8 years. As before, the statistic computed was the Pearson *r*.

The maxillary coefficients from correlating width at age 4 years with width at age 8 years are 0.93 for the 40 males and 0.90 for the 37 females. Comparable mandibular *r*'s of 0.90 and 0.91 were obtained. Generalizing, in both dental arches there is a moderately high association between interbuccal width of the deciduous second molars at age 4 years and the same width 4 years later. The index of prediction efficiency for $r = 0.91$ approximates 60 per cent.

Pearson *r* results from correlating width of arch at age 4 years with change in width of arch during the period from 4 to 8 years are as follows: for the maxillary arch, 0.13 and –0.08 on males and females, respectively; and on the mandibular arch, –0.09 and –0.011 on males and females, respectively. In other words, no statistically significant association is found between interbuccal width of the deciduous second molars at age 4 years and change in this width over the succeeding 4-year age span.

Relations of Arch and Face Widths.—Relationships were studied for (1) maxillary arch width with width of the upper face, and (2) mandibular arch width with width of the lower face. The data on upper face width, bizygomatic diameter, were secured by direct measurement; the data on lower face width, bigonial diameter, from measurement of posteroanterior roentgenograms. Detailed descriptions of both procedures, including information on their reliabilities, have been published elsewhere.[24, 25]

Pearson correlation coefficients were calculated on the 40 male subjects measured at age 6 years. For bizygomatic diameter and maxillary interbuccal width at the deciduous second molars $r = 0.37$, for bigonial diameter and mandibular interbuccal width at the deciduous second molars $r = 0.40$. Both coefficients are statistically significant at the 5 per cent level of confidence.

Several previous studies of the association between widths of the maxillary arch and width of the upper face are reviewed in a paper by Meredith and Higley.[26] Representative findings for the childhood years are: $r = 0.33$ for bizygomatic diameter with width at the deciduous first molars on 98 children 2 to 5 years of age, $r = 0.33$ for bizygomatic diameter with width at the deciduous second molars on 34 females 5 years of age, and $r = 0.38$ for bizygomatic diameter with width at the permanent first molars on 342 males 8 to 14 years of age. On the whole, results to date indicate that throughout childhood the efficiency of predictions of arch width from measurements of face width is less than 10 per cent.

SUMMARY

Major sections of this report treat: reliability of arch width measurements taken between the buccal aspects of the deciduous second molars; age and sex differences in width of the dental arches; growth of individuals from 4 to 8 years of age in maxillary and mandibular arch width; and relationships between arch size, arch change, and face size.

The subjects were 77 North American white children. Each had, throughout the 4-year period studied, 8 erupted deciduous first and second molars free from restorative dental work and orthodontic treatment.

Selected findings are:

1. Maximum transverse diameter of the dental arches at the deciduous second molars can be determined with high dependability. Reliability coefficients approximate $r = 0.995$, and differences between independent records rarely exceed 0.2 mm.

2. Interbuccal width at the deciduous second molars differs with arch, sex, and age. On the whole, the maxillary arch is 3.1 mm. wider than the mandibular arch, the male arches are 1.9 mm. wider than the female arches, and the dental arches at age 4 years are 1.7 mm. narrower than at age 8 years.

3. The maxillary dental arch is slightly more variable than the mandibular dental arch. Typifying standard deviations are 2.3 mm. for the maxillary arch and 1.9 mm. for the mandibular arch.

4. Changes in arch width between 4 and 8 years of age differ widely from child to child. Some arches are no wider at 8 years than at 4 years, while others increase as much as 3.5 mm. in this 4-year interval.

5. The two dental arches are positively related with regard to both size at a given age, and change in size with advance in age. The association between width of the two arches at age 4 is represented by $r = 0.8$, that between width change in the two arches from 4 to 8 years by $r = 0.7$.

6. There is a moderately high relationship between width of a given arch at 4 years and width of the same arch at 8 years, but there is no relationship between width of a given arch at 4 years and change in width between 4 and 8 years. The r values approximate 0.9 and zero, respectively.

7. Correlations between dental arch widths and widths of the face are positive but fairly low. The r's lie close to 0.4 for both maxillary arch width with bizygomatic diameter and mandibular arch width with bigonial diameter.

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THE CORRELATION OF THE INCLINED PLANES OF THE ARTICULAR SURFACE OF THE GLENOID FOSSA WITH THE CUSPAL AND PALATAL SLOPES OF THE TEETH

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INTRODUCTION

THE assumption that a relationship exists between the articular tooth surfaces and the articular plane of the temporomandibular joint is usually made. Complete and partial dentures are designed on this basis.[1-3] Contrary to this point of view is the observation that the teeth do not contact during mastication.[4]

From the evolutionary point of view postural changes are possible underlying factors for the formation of the human fossa mandibularis.[5] Studies of the masticatory muscles[6, 7] as well as of the histology of the temporomandibular joint suggest that the joint is subject to functional stresses.[8] Although the form of the cranial bones may be modified through masticatory muscular function, evidence for a role played by the dental structures in cranial form is inconclusive.

It appeared that if exact measurements of the joint and tooth surface inclinations were made, the resulting data could be correlated so as to secure evidence relating to this problem. The results of these studies are presented below.

PART I

In order to determine whether a correlation exists between the inclined planes of the articular slope of the glenoid fossa and the cuspal and palatal inclined planes of the teeth of skulls, a measuring device was constructed as described below. The instrument consisted of 2 parts, a headholder and a measuring device.

A. *Headholder (Craniophore).*—A facebow with adjustable ear rods was attached to the clasps of 3 test tube holders. Two of the clasps were screwed to the ear rod tubes of the facebow. The third was attached to the curvature of the bow. All 3 clasps were adjusted to the same height, so that the facebow was fixed in a horizontal plane. This combination of facebow and test tube holders met the requirements of an anthropometric "open craniophore"[9] (Figs. 2 and 3).

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B. *Measuring Device.*—An instrument was constructed* to measure in millimeters the 3 dimensions of the glenoid fossa and the dental cusps (Fig. 1).

The base (A) consists of a square, heavy metal frame. In its parallel sides a ledge accommodates a sliding platform which is regulated by means of a screw attached both to the frame and to the platform (Fig. 1, *A*). The distance traversed by the platform is registered in millimeters on a ruler attached to one of the sides of the frame.

Fig. 1.—Plane recorder.

From the center of the sliding platform a vertical rod (Fig. 1, *B*) is attached at right angles to the base. The rod is long enough to allow sufficient room for the accommodation of different sizes of skulls in the headholder.

A graduated horizontal bar (Fig. 1, *C*) is attached at right angles to the free end of the rod. Along this horizontal bar a graduated pointer (Fig. 1, *D*) slides in a tube attached to a small platform. This platform is at right angles to the graduated horizontal bar and slides along it. The vertical pointer (*D*), marked in millimeter units, moves up and down freely.

The 3 parts of the device are attached to each other in such a manner that they move in 3 dimensions: The platform (*A*) moves back and forth; the graduated pointer (*D*) moves from right to left and up and down. Any amount of movement in these 3 directions can be read off in millimeters and can be recorded on millimeter graph paper.

*Parts of a gnathostatic surveillor were used.

METHOD

A. *Fixation of the Skull.*—With the norma basilaris up, the skull is mounted on the craniophore by means of the ear rods inserted into the pori acustici externi. The ear rods are at an equal distance from the mid-sagittal plane. The curvature of the facebow embraces the region of the occipital bone, leaving the facial structures open and easily accessible (Fig. 2). The third point determining the Frankfort horizontal plane, to which all skulls are oriented, is established by means of an anatomic pointer (Fig. 1*E*) fixed at the same height as the ear rods. The pointer touches the most inferior point of the

Fig. 2.—Plane recorder with pointer in position to measure articular surface.

infraorbital ridge (orbitale) (Figs. 2 and 3). The Frankfort horizontal plane remains fixed at the same level for all skulls, so that registrations in the vertical dimension for different skulls may be compared.

B. *Fixation of Plane Recorder.*—After the skull is mounted in the craniophore, the measuring instrument, which is referred to as a plane recorder, is brought to the skull from its ventral side in such a way that the horizontal bar (*C*) lies in parallel plane with the plane formed by the 2 ear rods passing

through the external acoustic meati (Figs. 2 and 3). Thus a stable and fixed relationship between the two units of the setup is established: (1) Both craniophore and plane recorder stand on the same horizontal plane (in this case the table top); (2) By paralleling the horizontal bar with the plane passing through the ear rods, the mesiolateral movements of the graduated vertical pointer remain perpendicular to the sagittal plane; and (3) Since the horizontal bar is at right angles to the sliding platform, the anteroposterior movements of the platform are parallel to the sagittal plane.

Fig. 3.—Plane recorder with pointer in position to measure dental cusps.

Measuring the inclination of the fossa (Fig. 2): The measurements of the anterior wall of the glenoid fossa are carried out anteroposteriorly and lateromedially, i.e., (a) starting distally to the zygomatic arch near the articular eminence, moving backward toward the floor of the glenoid fossa, along the articular surface of its anterior wall, and (b) moving in the direction of the midsaggittal plane. Lateromesial measurements are taken at two millimeter intervals, giving cross-section registrations of the articular eminence, anterior slope,

and fundus of the glenoid fossa. On the average, 8 to 9 cross-sectional measurements at 2 millimeter intervals are necessary to determine the slopes of the entire fossa from lateral to medial (Fig. 5).

Assuming that the graduated vertical pointer is at the most ventral point of the eminence, and medial from the most lateral point of the articular eminence, the distance is read off in millimeters on the vertical pointer, and recorded on millimeter graph paper. It is related to the horizontal coordinate accepted as the projection of the Frankfort horizontal plane. By turning the screw, the sliding platform (Fig. 1, *A*) is then moved 1 millimeter from anterior to posterior bringing the vertical bar into a new relationship with the fossa.

Fig. 4.—*AP* = anteroposterior cross section of glenoid fossa; *d-f* = direction of movement of sliding platform; *cd* = initial position of vertical pointer; *ef* = final position of vertical pointer; *2* through *13* = intermediate positions of vertical pointer at 1 mm. intervals.

The distance in 2 (Fig. 4) is obviously larger than in 1 since the vertical pointer slides down on the incline of the anterior wall as the sliding platform is moved distally. This distance is recorded on the graph paper, using the method of finding a point in the coordinate system when the values of the abscissa and the ordinate are known.

The sliding platform is moved distally 1 millimeter after the recording of each point until the deepest point of the floor of the fossa is reached. The different points thus obtained at 1 millimeter intervals are then connected. The resulting sigmoid curve is the exact projection of the measured cross section of the articular surface. Then the vertical pointer is moved 2 millimeters mesially toward the mid-sagittal plane and the entire procedure is repeated for another cross section. Repeated measurements of this kind give a full picture of the anterior fossal incline in a number of cross sections (Fig. 5).

Dental measurements (Fig. 6): The measurements of cuspal slopes of teeth are carried out in the same way as those for the fossa, and are recorded in the same manner. Three measurements at 1 millimeter intervals are sufficient to determine each cuspal slope. The same procedure is repeated on the opposite side of the skull.

In order to establish the angulation of the inclined planes, the following technique is applied: (1) For the fossa, a tangent to the slope between the 2 points of inflection is drawn. The angle between the tangent and the horizontal

registration plane gives the angle of the inclination of the slope of the anterior wall of the glenoid fossa; (2) For the teeth the line recorded by means of the measurements of the cuspal and palatal inclined planes is extended. The angle formed between the extension and the plane of registration (abscissa) gives the angulation of the inclination of the cuspal and palatal slopes.

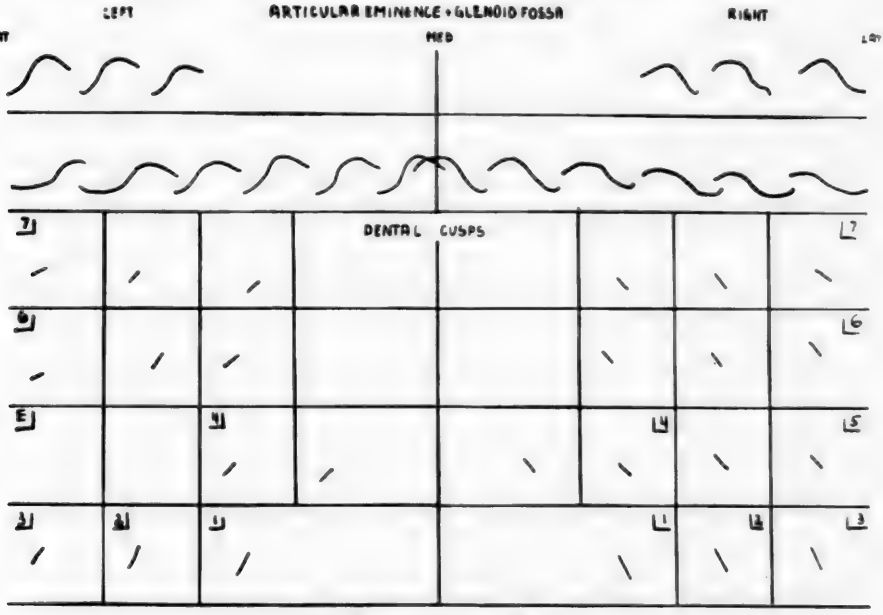

Fig. 5.—Registration chart.

PART II

MATERIAL

The investigation was carried out at the Department of Anthropology, Museum of Natural History, in New York. Ten skulls, taken from the Hungarian group of Demko-Hegy dating back to the 12th Century, were selected for this purpose. Only skulls with normal occlusion were selected; the cusps of the teeth were nonabraded or worn to a negligible degree. The chronologic age was determined roughly. Seven of the skulls were of adults up to approximately 35 years of age, 2 skulls belonged to children above 7, 1 to a child above 12. The degree of overbite was noted and fell in the range of 1 to 5 millimeters. Sex was not taken into consideration in this study. Within the limitations of these controls the skulls were chosen at random. The measurements were limited to the glenoid fosa and the maxillary teeth.

METHOD

The measurements included the following: (a) the articular slope of the glenoid fossae (Fig. 2), and (b) dental measurements (Fig. 3); (1) molars—The slopes of the distobuccal, mesiobuccal, and mesiolingual cusps of the first

and second molars were measured. Third molars were not taken into consideration; (2) premolars—The buccal and lingual cuspal inclines were measured; and (3) second incisors and first incisors—On canines, the palatal slope was measured.

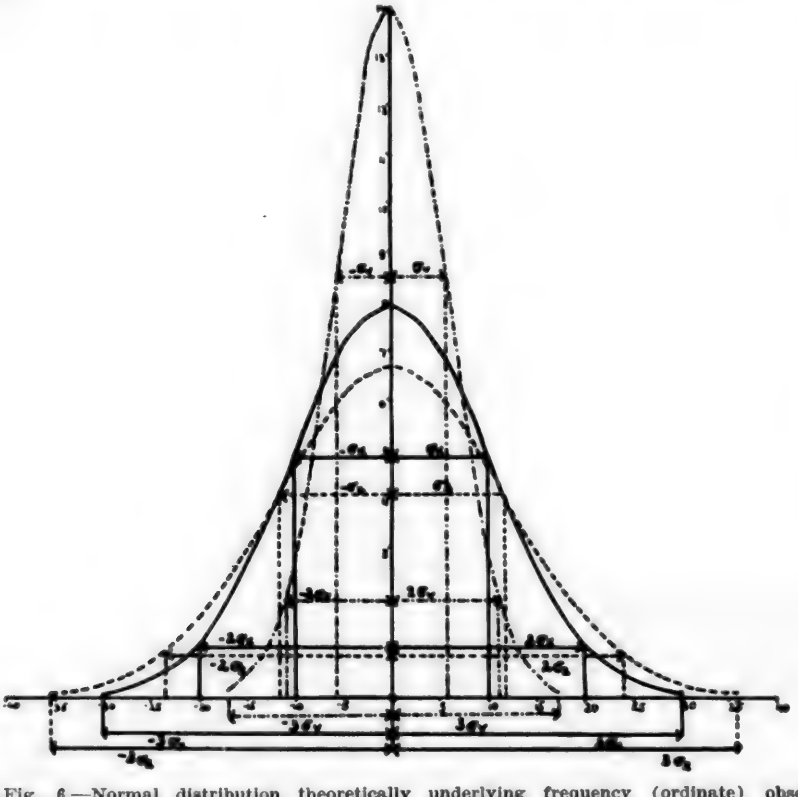

Fig. 6.—Normal distribution theoretically underlying frequency (ordinate) observed values standardized to mean of zero (abscissa). x = solid line = observation on glenoid fossa; z = dashed line = observations of the anterior teeth; v = dot-dash line = observations of the posterior teeth.

The collected data (Table I) were subjected to statistical analysis.*

RESULTS

The following dimensions were determined: the slope of the anterior wall of the glenoid fossa; the cuspal and palatal inclination of all the teeth (posterior and anterior); and then separately, the cuspal inclination of the posterior teeth (molars and premolars); and the palatal slope of the 6 upper anterior teeth.

*Ten skulls were measured, but all the computations were done on the basis of 20 observations, since the left and right fossae and the teeth of each respective side of the skulls were computed separately: (1) The average slope for each fossa was found; (2) The average slope for each tooth was determined; (3) From these, the averages for the molars and premolars was calculated; (4) The averages of the palatal slope of the upper anterior teeth were calculated; and (5) The averages of all teeth taken together, i.e., the average from the second molar to the central incisor for each respective side (left and right) was calculated. These were the basic figures with which the statistical analysis was carried out.

TABLE I

MASTER CHART OF AVERAGES*

SKULL NO.	SIDE	AVERAGE CUSPAL INCLINATION (y)	AVERAGE PALATAL SLOPE (z)	AVERAGE CUSPAL SLOPE OF POSTERIOR TEETH (v)	AVERAGE ANTERIOR SLOPE GLENOID FOSSA (x)
1	L	38	41	34	46
	R	36	41	33	56
2	L	40	45	32	42
	R	41	45	38	51
3	L	31	33	29	30
	R	29	28	31	27
4	L	46	57	41	57
	R	47	55	43	60
5	L	40	46	36	52
	R	48	55	43	44
6	L	35	26	44	45
	R	38	38	28	41
7	L	42	49	37	63
	R	45	50	42	51
8	L	31	26	35	43
	R	34	28	40	58
9	L	27	31	30	32
	R	32	35	30	46
10	L	51	60	38	59
	R	56	65	49	49

*Units in degrees.

By analyzing the averages of the articular slopes of the fossae it was found that they have a comparatively large range of variation with a standard deviation of 10.1 degree from the mean of 47.6 degrees (Table II). Moreover, the 2 fossae on 1 individual skull seldom have the same mean inclination (Table I).

The average slopes (cuspal and palatal) of the teeth, however, show less variation than the fossae, having a standard deviation of 7.91 degrees with a mean of 39.35 degrees, and less intracranial variability (Tables I and II).

TABLE II

VARIABILITY OF THE MEASURED UNITS

	1		2	3	4
	SLOPE				
UNIT	MIN.	MAX.	RANGE	MEAN	STANDARD DEVIATION
Fossa	27	63	36	47.6	10.1
(left and right)	3	14	11		
All teeth	27	56	29	39.35	7.91
(left and right)	1	8	7		
Posterior teeth	28	49	21	36.65	5.7
(left and right)	0	16	16	L: 35.6	
	1	8	7	R: 37.7	
Anterior teeth	26	65	39	42.7	11.96
(left and right)	0	12	12	L: 41.4	
	2	9	7	R: 44.0	

The study of the 2 separate dental groups (posterior and anterior) shows that the variability of the palatal slopes is greater (standard deviation 11.96 and mean 42.7) than the variability of the cuspal slopes of the posterior teeth (standard deviation 5.7, mean 36.65) (Fig. 7).

Comparing the mean of the averages of the anterior slope of the fossae to the mean of the averages of the cuspal and palatal inclinations of all the teeth, the correlation coefficient was found to be 0.597 and significantly different from zero.* In order to find whether the posterior teeth or the anterior teeth account for the preponderance of the relationship between the inclines of the articular

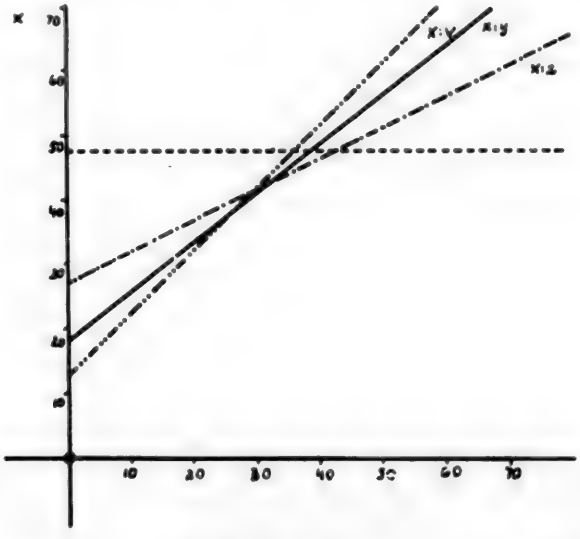

Fig. 7.—Composite diagram of regression lines. $x:y$ = line of regression for the anterior fossal slope (x) based on the values of the average dental slopes (y); $x:z$ = line of regression of the anterior fossal slope (x) based on the values of the palatal slopes of the upper anterior teeth (z); $x:v$ = line of regression of the anterior fossal slope (x) based on the values of the cuspal slopes of the maxillary posterior teeth (v).

surface of the glenoid fossa and the functional slopes of the teeth, the finding for the dental slopes were divided into 2 groups: contained the values for the cusps of the posterior teeth, and the other contained those for the palatal slopes of the 6 upper anterior teeth. The correlation coefficient for these 2 groups and the articular slope of the glenoid fossa were 0.549 and 0.535, respectively (Table III, column 1). These values, too, were found to be significantly different from zero.†

The slopes to the regression lines of the scatter diagrams were (Fig. 7) found to be 0.75 for the measurements of all the teeth, 0.47 for the palatal slopes of the upper anterior teeth, and 0.95 for the cuspal incline of the posterior teeth, as related to the measurements of the articular slope of the glenoid fossa. All of them were found to be statistically different from zero.‡

*To test the significance of difference between 2 means the T-test for small samples was used (Fisher,[10] p. 122.).

†The T-test was used and the criterion of significance taken at the 0.5 level. It may be noted that the correlation coefficient of 0.597 is also significant at the 0.01 level.

‡Using the T-teat the slopes of the regression lines were all found to be significant at better than the 0.001 level. The slope of 0.751, for example, is to be interpreted as follows: to each 1 degree increase in the dental slope corresponds ¾ of 1 degree increase in the articular slope of the fossa.

TABLE III

VALUES FOUND IN REGRESSION ANALYSIS

CORRELATION BETWEEN		1	2	3	4
		r	b	$S_{1, 2, 3}$	S_b
1	xy	0.597	0.75	3.83	0.1166
2	xz	0.549	0.47	4.37	0.08368
3	xv	0.535	0.95	4.47	0.17627

1, r = correlation coefficient.
2, b = slope of regression line.
3, $S_{1, 2, 3}$ = standard error of estimate (scatter around regression line).
4, S_b = estimated standard deviation of slope of the regression line.

DISCUSSION

The variability of the articular slope of the glenoid fossa was found to be considerably large, both within the limits of the sample group analyzed and within each individual skull, the left and right sides differing from each other. Disregarding the range of variation between the 2 fossae within 1 skull, it might be assumed that the large deviation from the mean value found for the articular slope is due to an individually inherited genetic pattern for the articular slope. The fact, however, that a large range of variation was found between the left and right sides of the fossal slopes may bring further evidence that the fossa is subjected to functional stresses, and that those stresses have an influence in the modification of the genetic pattern of the articular slope.

In contrast to the findings for the glenoid fossa the slopes of the teeth seem to exhibit a greater constancy in their inclined planes; the range of variation and their standard deviation is still sufficiently high to indicate considerable individual variation. In comparing the cuspal and palatal inclinations of the teeth in the left and right side of each skull, it was found that 70 per cent of the observations show a minimal difference of 1 to 3 degrees only. At this point it should be mentioned that the measurements obtained for the dental inclined planes were not registrations of their true anatomic slopes, but rather of their functional inclines, since the individual position of each tooth in the dental arch and the curve of Spee modify the angulation of their anatomical slopes. From this observation the tentative inference could be drawn that, in cases with normal occlusion, the factors which influence the position of the teeth in the dental arch are more constant and uniform than those which are involved in the modification of the articular slope of the glenoid fossa.

All the applied methods used to determine whether a correlation between the articular slope of the fossa and the dental inclined planes existed proved to be positive and significant at the 0.05 level. Yet all the computations of the values found for the articular slope of the fossa against the composites of the dental inclines indicate that the articular fossal slopes exhibit closer correlation to the palatal inclined planes of the anterior teeth than they do to the cuspal slopes of the posterior teeth. It was found that no significant difference existed between the average slope of the anterior fossal slope and that of the mean palatal slope of the anterior teeth (47.6 and 42.7 degrees, respectively), while the cuspal slope of the posterior teeth (36.65 degrees) was different from the

average fossal slope. The correlation coefficient is also higher for the relationship between the fossa and the anterior teeth (see Table III). Furthermore, it was found that the slope of the regression line for the articular slope of the fossa based upon the observations of the palatal slopes of the anterior teeth is more reliable than that for the cuspal slopes of molars and premolars.

The higher correlation found between the articular slope of the glenoid fossa and the palatal slope of the upper anterior teeth could suggest a possible functional relationship between these two units, due to the protrusive movements of the mandible. It was surprising to find that the slope of the anterior wall of the fossa showed a smaller correlation to the posterior teeth. This finding is in contradiction to Gysi's statement that protrusive balance is caused by the inclined planes of the molar cusps as well as by the curve of Spee. It would be interesting to correlate the left posterior measurements with the right fossa and vice versa in order to find the results for lateral movement.

It should be emphasized, however, that the measures of correlation and of the slopes of the regression line, although significantly different from zero at the 0.05 level should not be accepted uncritically. Even the regression line related to the highest correlation coefficient—0.587 for the composite (total) dental slopes—"explains" considerably less than half (36 per cent) of the variations in the slopes of the anterior wall of the fossa from their mean.* Perhaps more relevant in relation with the comparatively low correlation coefficients obtained is the fact that the slopes of the anterior or posterior teeth together with some other important factors modify the articular slope of the fossa. It is undoubtedly logical to consider the muscles of mastication as one of the most important factors. Consideration should also be given to the form of the articular disc, and maybe to the size of the articular eminence.

The correlation found between the slopes of the fossa and the slopes of the teeth is, however, significant enough to make further and more extensive investigations in this field necessary; at this point no positive or negative conclusions can be made.

SUMMARY

1. The construction of a measuring device and the method of recording anatomic structures are described.

2. The articular slope of 20 fossae and the cuspal and palatal slopes of the maxillary teeth in 10 skulls were studied in order to determine if any correlation between these units existed. The skulls selected had to conform with the following requirements: normal occlusion, nonabraded cusps, and an amount of overbite between 1 to 5 millimeters. Within these limitations of control, the skulls were chosen at random.

3. The anterior slope of the fossa and the inclined planes of the cusps and palatal slopes were measured and recordings made. Their slopes were determined in units of degrees.

*A correlation coefficient of 0.5 would explain only 25 per cent of the variation (Garrett,[11] p. 74.).

4. The collected data were subjected to statistical analysis which gave the following results: (a) The anterior slope of the glenoid fossa shows great individual variation both within the sample group and within each skull; (b) The teeth exhibit a greater constancy in their inclined planes and show less variation between the two sides of one and the same skull, although the palatal slopes of the upper anterior teeth show greater individual variations than the cuspal slopes of the posterior teeth; (c) No significant difference was found between the main slope of the articular surface of the glenoid fossa and this of the palatal slope of the upper anterior teeth, although the mean cuspal slope was found significantly different; (d) The correlation found between the articular slopes of the fossae and the dental slopes (both cuspal and palatal) appears significantly different from zero at the 0.05 level; (e) All the statistical methods applied seem to indicate a closer relationship between the articular slope of the fossa and the palatal slope of the upper anterior teeth, the correlation to the cuspal inclines of the posterior teeth being less significant; and (f) On the basis of this study alone no definite conclusions can be made if and to what extent the teeth have a functional influence upon the configuration of the articular surface of the glenoid fossa.

I wish to express my thanks and appreciation to Drs. Arthur Toten and Armand Oppenheimer, of the Department of Orthodontics, and Dr. Barnet Levy of the Department of Dental Research at Columbia University for their advice, helpful suggestions, and encouragement, to Dr. Harry L. Shapiro, Curator of the Museum of Natural History, New York, for making the skull collection available, and to Edward Kilberg for his assistance in carrying out the statistical analyses.

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VARIABILITY OF THE FORM OF MAXILLARY PERMANENT INCISORS

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A COMMON clinical observation is that upper incisor teeth differ considerably in form from one person to another, and also between racial types, e.g., English and Negro, but little data has as yet been accumulated on this subject. It was therefore considered worthwhile to undertake a brief study of incisor tooth form with the object of stating, with some precision, these differences which might be of value for research in both genetics and anthropology.

The maxillary first permanent incisors are suitable for this study because of their simple shape and relative constancy (i.e., lack of malformations or extra cusps, etc.). Wheeler[15] stated that, "They are also the most representative of the mold design of the teeth, . . . when development has been normal all the teeth are in harmony with the central incisor." Seipel[14] added weight to this observation when he calculated the coefficients of correlation between the maximum mesiodistal widths of these and the other teeth of the upper jaw, and found (with the exception of the first permanent molar) that they were all over 0.5. Finally the essential shape of this tooth is laid down before, or just after birth (Schour and Massler[13]) in the stage of morphodifferentiation. This is when the form of the amelodentinal junction is mapped out, the enamel merely being laid on it incrementally as shown by the relatively constant width of enamel in widely differing shaped teeth (Fig. 4.) (See also Diamond and Weinmann.[6]) It may then be assumed that environment can have little effect on incisor tooth form and size, and, in fact, they are primarily determined genetically.

Williams[9] classified the shape of first incisors into 3 types: tapering, square, and ovoid, and stated that they appeared in all races of man, and in the anthropoid apes. Azouley and Regnault[2] measured the width of a number of maxillary first incisors of different races and of chimpanzees and gorillas. They calculated an index which was obtained by subtracting the width at the neck of the tooth from the width at the incisal edge. This gives a measure of the taper, i.e., the way in which the mesial and distal sides of the tooth converge. They found that apes had the highest index (greatest taper), then Negroes, Australians, Chinese, Europeans, and finally Indians. They conclude that tapering teeth are more common among the "primitive races" than among the more civilized. Ashton and Zuckerman,[1] in an extensive investigation of the size of teeth in modern and fossil apes, measured a number of dimensions for every tooth, and compared each separately between the different

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groups to find whether there were any significant differences. Bronowski and Long[4] have criticized this method on the grounds that a number of separate measurements of an object does not adequately specify the whole and suggested that it would be better, when comparing 2 different groups, to combine the set of measurements (e.g., crown height, width at neck, width at the incisal edge, and depth) into a discriminant function of the type $X = a_1x_1$ and a_2x_2 and a_3x_3 and a_4x_4, the coefficients being chosen to maximize the differences between the two groups.

METHOD

In order to present the results in a quantitative way as well as bringing out obvious differences in shape it seems a new approach is needed. If the labial aspect of the crown of the maxillary first permanent incisor could be regarded as a trapezium, i.e., by ignoring its curvatures, then its shape could be adequately described by three values: (1) surface area—an estimate of overall size, (2) width index—a relation of the width to the height, and (3) taper—a measure of the convergence of the mesial and distal sides.

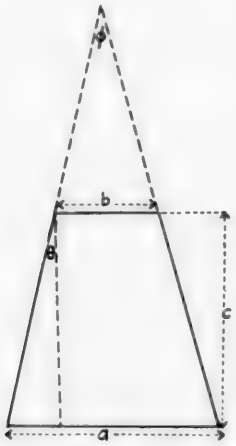

Fig. 1.—*a*, Maximum mesiodistal width; *b*, Cervical width; *c*, Maximum crown height; ϕ, Angle of taper ($= 2\theta$).

Fig. 1 shows an outline of such a trapezium wherein a is the maximum mesiodistal width (between approximal surfaces parallel to the labial and incisal surfaces); b, the cervical width taken parallel to a at the neck of the tooth; and c, the crown height.

The surface area (mm.^{2}) is then $\frac{c}{2}(a+b)$, the width index $100 \times \frac{a}{c}$, and the taper $\emptyset$ degrees. ($\emptyset = 2\theta$ and $\tan \theta = \frac{\frac{1}{2}(a-b).}{c}$)

MATERIALS AND RESULTS

A sample of 50 extracted modern English teeth was obtained from the General Anaesthetic department of the Birmingham Dental Hospital (not

separated for sex or side), and since they were selected at random it was considered they would be sufficiently representative.

These were measured by means of specially made vernier calipers reading to 1/50 mm., and the 3 values previously described were calculated for each tooth.* In Table I are recorded the means, standard deviations, and ranges for these results.

Another method of presentation to indicate the variability better is shown in Fig. 2, where the values of surface area in mm.² have been plotted on arithmetic probability paper (a straight line has been drawn through these points, presuming a normal distribution). Various arbitrary limits may now be selected and the proportions of results falling in each found, e.g., 20 per cent results are below 80 mm.², 73 per cent results are from 80 to 100 mm.², and 7 per cent results are above 100 mm.².

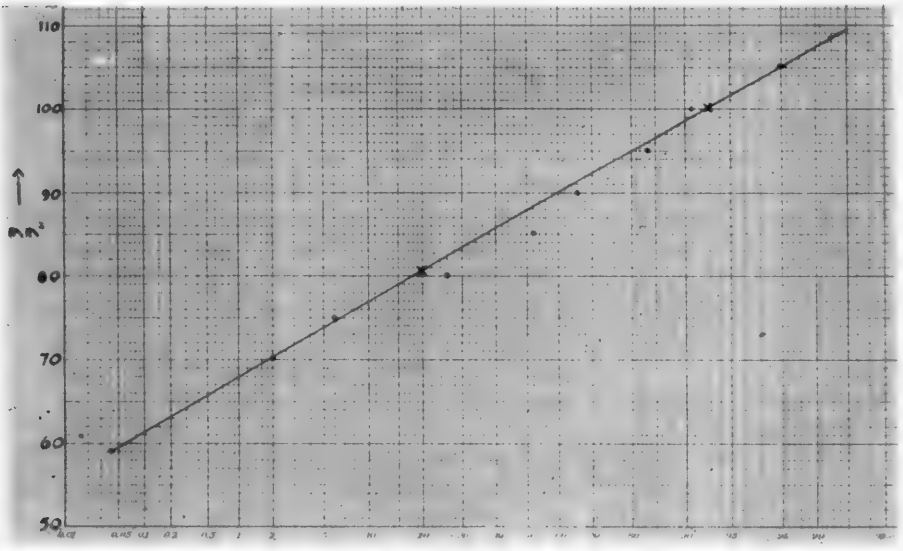

Fig. 2.—Surface area of labial aspect of crown of maxillary permanent first incisor (mm.²). (Modern English.)

A search through the literature of tooth measurement indicates that few investigators record the cervical widths of maxillary first incisors, and so there are not many results that can be compared with the present ones. However, those available are recorded in Table II. The Saxon results were obtained by the author measuring 36 teeth from skulls in the Bidford-on-Avon collection at the Birmingham University (applying the same correction for attrition which was more frequent in these teeth). These skulls were obtained from a sixth century burial ground about 25 miles from Birmingham

*A correction for height was applied in cases of attrition. If mild, 1 mm. was added to the recorded height; if moderate, 2 mm.; and if severe the tooth was rejected.

(Humphreys[8]), and so represent the people who lived in much the same geographical location as the modern English sample, some 50 to 60 generations earlier.

TABLE I
SAMPLE OF MODERN ENGLISH TEETH (N = 50)

	SURFACE AREA (MM.²)	TOOTH WIDTH INDEX	TAPER (DEGREES)
Mean	86.83	73.21	7.90
Standard deviation	8.94	5.88	2.26
Range	69.7-116.0	60.1-88.7	2.9-13.7

TABLE II
COMPARISON OF MODERN ENGLISH TEETH WITH OTHER RACIAL GROUPS

TYPE	TOOTH WIDTH INDEX	TAPER (DEGREES)
White American[3]	90.0	15.3
Bantu[10]	84.8	14.1*
American Indians[12]	87.8	not possible
Japanese[11]	71.2 ♂	not possible
	73.6 ♀	
Modern English	73.2	7.9
Saxon	75.9	8.7
Chimpanzee[1]	100.5	15.2*

Surface area has been omitted owing to lack of sufficiently accurate, comparable data.
*Cervical width estimated from Azouley and Regnault's indices of appropriate types.

DISCUSSION

The crown of the maxillary first permanent incisor was chosen for analysis because of its simple and relatively stable shape and also because it is representative of the general mold of the teeth. However, the analysis could be extended to other teeth by means of coordinate transformation (D'Arcy Thompson[5]). This method is merely one in which the normal x and y coordinates are altered, e.g., by lengthening the interval between them, or curving one and not the other, etc. In Fig. 3 the dotted outline gives approximately the shape of an upper canine tooth and superimposed on it (*a*) is

TABLE III

			WIDTH INDEX								
			A (-85)			B (85-95)			C (95-)		
SURFACE AREA (MM.²)			A (-80)	B (80-95)	C (95-)	A	B	C	A	B	C
(-1°) A	Taper	♀	1 (2.1)	1 (1.9)	2 (0.4)	10 (9.8)	9 (8.9)	0 (1.8)	8 (5.9)	5 (5.3)	1 (1.1)
		♂	1.5 (3.9)	6 (7.5)	9 (3.9)	6 (5.3)	9 (10.3)	6 (5.3)	1.5 (1.6)	1.5 (3.1)	1.5 (1.6)
(1-3°) B		♀	0 (2.6)	3 (2.3)	2 (0.5)	8 (11.9)	17 (10.7)	2 (2.2)	6 (7.1)	5 (6.4)	2 (1.3)
		♂	4.5 (4.5)	12 (8.8)	1.5 (4.5)	9 (6.3)	12 (12.1)	3 (6.3)	1.5 (1.9)	4.5 (3.7)	1.5 (1.9)
(3°-) C		♀	2 (1)	1 (0.9)	0 (0.2)	8 (4.8)	1 (4.3)	0 (0.9)	5 (2.8)	1 (2.5)	0 (0.5)
		♂	0 (0.8)	1.5 (1.6)	0 (0.8)	1.5 (1.1)	3 (2.2)	0 (1.1)	0 (0.3)	0 (0.7)	3 (0.3)

Per cent of each measurement in each group.
Expected values in parentheses. (See text.)

the result of plotting the same outline, but using one curved and one normal coordinate. In *b* the one coordinate only is increased progressively downwards by the addition of 1 mm. every centimeter. As may be seen, these transformations alter surface area, tooth width index, and taper, and it should be possible in this way to establish a series of standards with which a given tooth might be compared.

The variability of the 3 characteristics can be seen from their ranges (Table I) to be considerable and various ways might be used to bring this out; for example if surface area, tooth width index, and taper were each divided into 3 groups, then 27 possible combinations would occur and provide a convenient way of typing a tooth. Table III shows how this is set out in a 3-way table (unpublished data on school children with "expected" values, on the assumption of independence of the 3 characters, in parentheses [Goose[7]]).

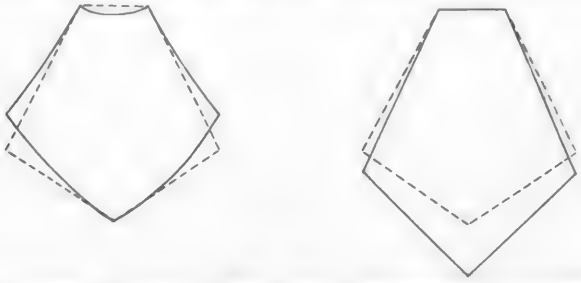

Fig. 3.—Outlines of canines, dotted lines; transformations, unbroken lines.

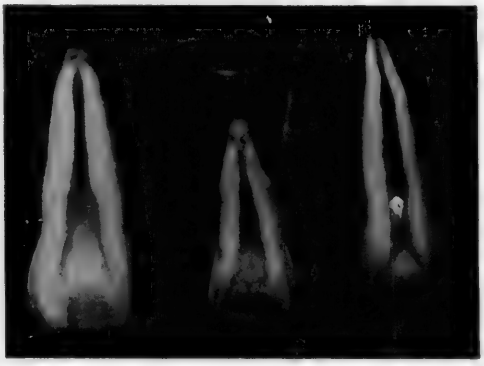

Fig. 4.—Labial surface of permanent maxillary first incisor. (×3/2.)

This might facilitate the study of inheritance of tooth form and size, and perhaps help solve such problems as whether large teeth may be inherited from one parent and small jaws from the other.

Different racial types might be compared by mean values, or by the relative proportions of the possible 27 types, or some such similar classification. It is noticeable that the Bantu, American Indians, and white Americans have considerably wider (relative to crown height) teeth than the English or

Saxon groups, but the Japanese are rather similar. Also, it is obvious that English (and Saxon) teeth are rather straight sided compared with the other groups, and, to show a typical outline of an English tooth, the average measurements were plotted (Fig 5). Perhaps when further comparative data are available more significant conclusions may be drawn from these differences.

The chimpanzee teeth which have been included in the table for comparison show decidedly higher tooth width index which puts them in quite a different category to human teeth. Their taper also is high although not higher than the white American, and this seems to indicate that this latter result is incorrect. This may be due to Black's[3] figure for cervical width (6.3 mm.) being too small. It would give a value, for Azouley and Regnault's[2] index, of 2.7 mm.: the latter gave 1.61 mm. for Europeans, and I find my results give an average of 1.55 mm. In view of these findings it seems necessary to recalculate the angle of taper for white Americans using a corrected cervical width of 7.4 mm. (obtained by subtracting Azouley and Regnault's[2] index of 1.61 mm. from the mesiodistal width of 9.00 mm. [Black[3]]). The angle of taper is then 9.2 degrees which seems much more likely.

Fig. 5.—Outline of labial aspect of maxillary permanent first incisor. (Modern English.) (×5, reduced ½.)

Finally, it may be seen from Table II that Saxon and modern English teeth are quite similar in shape (their surface areas are also closely comparable) so that it appears that, over a period of 50 to 60 generations, incisor form and size have not altered materially in this region. This may strengthen the belief that the method of analysis described may be useful in anthropologic research into racial variations.

SUMMARY

1. A new method of measuring the shape and size of maxillary first permanent incisors has been suggested by using (1) the labial surface area, (2) tooth width index, and (3) the taper (angle at which the mesial and distal sides converge).

2. Fifty modern English teeth have been measured and it has been shown that the data may be presented either by mean and standard deviations, or in the form of groups read off from the distributions plotted on arithmetic probability paper, and then combined together in a 3-way table giving 27 different types.

3. The present results show considerable differences when compared with other racial groups in the literature, but similarities with my own Saxon data.

4. It is shown that the form of these teeth is little affected by environment, but is the result primarily of heredity and is therefore a suitable character for genetic analysis.

5. The method can be extended to other teeth by means of coordinate transformations, and an example is given.

I wish to thank Professors E. B. Manley and S. Zuckerman for permission to examine Saxon skulls in their collections, and also Dr. Marsland for his assistance in providing me with the modern teeth.

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THE RELATIONSHIP OF THE SEASON OF BIRTH TO THE STATE OF THE PRIMARY DENTITION

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IN THE course of routine inspections of school children it was noticed that the deciduous teeth of children born in the winter months were frequently inferior to those of children born in the summer. Although Mills[1] and Price[4] have shown that dental caries is more common in northern latitudes and is possibly connected with the amount of sunlight available, no other work could be traced which has a bearing on this observation which, if true, is of some importance in the epidemiology of dental caries. Accordingly such clinical records as are available were scrutinized and are presented.

The children described were 5-year-old entrants to the state schools in Herefordshire, and numbered 300 out of an estimated total of 2,000. They were for the most part born in 1947, a year remarkable for its extremes of weather. The sample was drawn from all parts of the county and is within the limits stated a random one.

DENTAL CARIES

The teeth were examined by inspection only using an illuminated spatula. The time given to each examination was unlimited, but neither probe nor x-ray was available. The extent to which individual teeth were affected was not evaluated. The teeth were recorded as "Present," "Carious," "Filled," or "Absent." The extent of caries was measured in terms of the D.M.F. as modified by Sandler[5] for use in connection with children of this age. It is referred to as d.e.f. in Tables I and III.

TABLE I
VARIATIONS IN THE INCIDENCE OF DENTAL CARIES IN CHILDREN ACCORDING TO THE SEASON OF THE YEAR IN WHICH THEY WERE BORN

PERIOD OF BIRTH	DEC., 1946 TO FEB., 1947	MARCH TO MAY, 1947	JUNE TO AUG., 1947	SEPT. TO NOV., 1947
Number of children examined	40	58	50	58
Children with <2 d.e.f.	12	20	25	24
Total d.e.f.*	184	194	170	225
Mean d.e.f.	4.6	3.3	3.4	3.9
S.D. of Mean	7.5	6.9	6.4	5.9

*$\chi^2 = N = 3$: P 0.01.

DENTAL STRUCTURE

Surface structure was noted and assessed for macroscopic lesions of enamel. Discolored incisors with a history of trauma were excluded as were

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6 cases of extensive caries in which the remaining enamel was insufficient for assessment. Children were separated into 2 broad classes of "Normal" or only very slightly imperfect, and "Defective" structure. The former corresponds to hypoplasia M type, Grades 0 and 1; the latter consisting of moderate and severe lesions in one or more teeth to Grades 3 and 4 described by Mellanby.[6] The plates included in this monograph were used as a guide in assessment. The findings are given in Table II. In 10 (3.2 per cent) of the children hypoplasia of the G type, as described by earlier writers, was found. The enamel lesions were of a more severe type but do not necessarily predispose to caries formation (King[7]).

TABLE II
THE EXTENT OF DEFECTIVE STRUCTURE

STRUCTURE	"NORMAL"	"DEFECTIVE"
No. children	250	64
Per cent of children	79.6	20.4

The association between caries and structure is shown in Table III.

Differences in the quality of teeth in samples of children born at different seasons in 1947 are shown in Table IV.

TABLE III
THE INCIDENCE OF CARIES IN DENTITIONS OF "NORMAL" AND OF "DEFECTIVE" STRUCTURE

STRUCTURE	"NORMAL"	"DEFECTIVE"
No. of children	250	64
No. of carious teeth	742	470
Mean d.e.f.	3.0	7.3
S.D. of Mean	2.9	5.0

WEATHER CONDITIONS

The year 1947 was one of extremes. The temperature records for February and March were the lowest since 1895, while the remaining 9 months all had temperatures above the average, particularly May (the highest since 1922) and August (since 1911). The total sunshine fell short of the average for the year by 86 hours. Only two months exceeded the average for sunlight, August and November.[3] These recordings made at the County Observatory accord with others made elsewhere in England and Wales at that time and published by the Meteorological Office.[2]

TABLE IV
VARIATIONS IN THE INCIDENCE OF DEFECTIVE STRUCTURE ACCORDING TO THE SEASON IN WHICH CHILDREN WERE BORN

SEASON OF BIRTH	1 DEC.-FEB. 1946-47	2 MARCH-MAY 1947	3 JUNE-AUG. 1947	4 SEPT.-NOV. 1947
Number of children examined	38	58	49	59
Number with defective structure	13	15	9	8
Per cent with defective structure	36	25	17.5	14.5

$\chi^2 = N = 3 : P = 0.05.$

Weather conditions which might possibly influence the development of the primary dentition include temperature and sunlight. Monthly variations of these were obtained from the County Observatory and are shown in Fig. 1. Here the percentage of children born in each month with normal structure is shown against curves for variations in sunlight and temperature. The figures

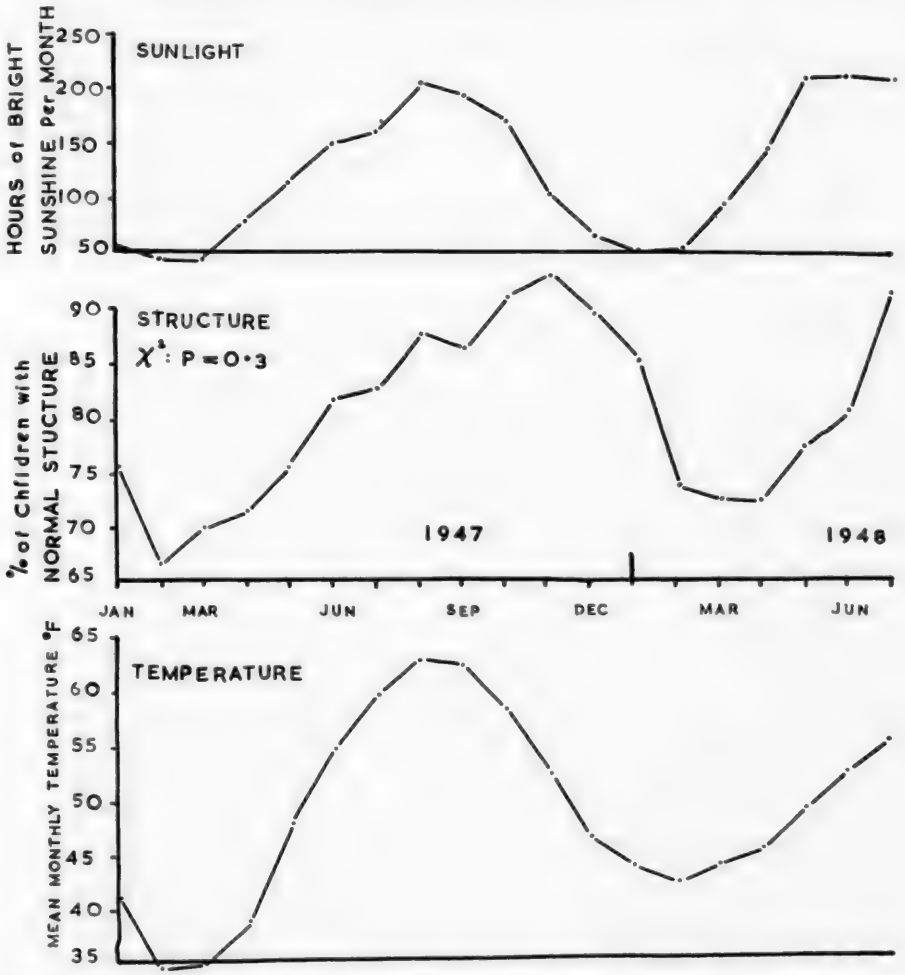

Fig. 1.—Monthly variations in temperature, sunlight, and dental structure. Figures are three-month running averages.

are presented as 3 months' running averages to smooth the curves. While the figures for structure are not statistically significant the curves present a striking similarity and, in fact, correlate at $r = 0.5$ for structure and sunlight and $r = 0.6$ for structure and temperature. The relationship appears to be rectilineal and the significance of the correlation is $P = 0.05$.

DISCUSSION

Taken at their face value the figures in Table I show that the teeth of children born in the winter months were, so far as visible caries is concerned, inferior to those born in the summer. In particular the total number of carious teeth found in each of the different seasonal groups showed a variation which is statistically significant, while other figures may well be fortuitous. The mean d.e.f. per child showed less distinctive differences between the groups than did structural defect in Table IV, possibly because of the influence of other factors such as diet, oral hygiene, and heredity.

The assessment of structure was made on broad lines because neither tactile methods nor a lens was employed. Figures for G hypoplasia are given separately as a matter of interest but were subsequently included in the class, "Defective Structure."

The number of children classified as having defective dental structure was probably low since some of the lesions were, no doubt, obscured by caries or the evidence removed altogether by previous extraction. The criterion of judging a mouth on a single tooth is also open to question but was adopted as probably the best in the circumstances.

While caries was found to be more common in children with defective structure the disparity between the two classes might have been greater if the normal group had been separated into hypoplasia, Grades 0 and 1. The probable omission of small cavities would have a similar effect.

When full account has been taken of a general tendency to underestimation the figures for caries and structure remain comparable and deductions are not necessarily invalidated on that account.

The value of correlations between climatic factors and structure and their statistical significance may be questioned since they are based on figures which are not themselves significant. It should be remembered, however, that failure to attain to significance indicates essentially a need for caution in drawing conclusions from such figures. Here the additional calculations serve to localize the weakness of the case, which is that the observations are not sufficient to support conclusive proof.

Nor, indeed, would this paper have been released at its present stage had opportunity for further work remained for the writer. The results of investigations are, therefore, offered not as fact conclusively proved, but as evidence sufficiently suggestive to encourage further investigation into the subject, preferably in a less temperate climate than that which normally pertains in the British Isles.

SUMMARY

Some 300 school entrants were examined, of whom those born between November, 1946, and November, 1947, showed a material variation in incidence of dental caries according to the season of birth.

The structure of the teeth was noted and teeth showing evidence of defective structure were found to have a considerably higher incidence of caries than those which did not.

The incidence of defective structure varied with the season of birth in 1947.

An association was demonstrated between the amount of defective structure and that of sunlight and temperature at or about the time of birth.

It is emphasized that the year reviewed is exceptional and the children are rural, permitting variations which might otherwise escape notice to become appreciable in a relatively small sample of children.

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ANTIBIOTICS IN OPERATIVE PROCEDURES AND ENDODONTICS—IN VITRO STUDIES

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ALTHOUGH antibiotics have been available for a decade, older medicaments are still commonly used for infected dentin sterilization in operative procedures and endodontics. The antibiotics possess more of the desirable qualities of an ideal agent and can be easily used where indicated.

Bartels and Nuckolls[1] reviewed the use of anti-infective agents in dentin sterilization and remarked on the lack of reports on the use of antibiotics. They proposed that the ability of topically applied antibiotics to penetrate into tissues and their relative lack of tissue irritation should make them highly suitable for the purpose and that they might prove to be the solution to the entire problem. A report by Hardwick[2] suggested, on the basis of experimental studies, that antibiotics might be applicable in infected dentin therapy.

The following report gives details of an in vitro study conducted to determine the effectiveness of several antibiotics, in various concentrations, against organisms present in dental infections. For the experiment 141 teeth, extracted because of advanced caries, were prepared and immersed in antibiotic preparations from 2 to 5 minutes, then removed, split, and cultures made of carious matter, shavings of contiguous dentin, and portions of the pulp. It was found that oxytetracycline (Terramycin) in a concentration of 250 mg. per cubic centimeter was the most effective sterilizing agent of the preparations tested. The bactericidal efficiency for each preparation was determined by the percentage of negative cultures among the total number of cultures made from all teeth immersed in it. The bactericidal efficiency per cent of the 14 preparations with their respective concentrations per cubic centimeter of solution is given in Table I.

With all of the preparations tested identification of organisms in positive cultures gave the following results in order of frequency: (1) from caries-streptococci, lactobacilli, and staphylococci; (2) from dentin-streptococci, staphylococci, and lactobacilli; (3) from pulp-streptococci, lactobacilli, and staphylococci. From control teeth without any medication, the following organisms were identified in order of predominance: (1) from caries-lactobacilli, staphylococci, and streptococci; (2) from dentin-staphylococci, streptococci, monococci, diplococci, lactobacilli, filamentous organisms, and leptotrichia; (3) from the

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pulp-streptococci and lactobacilli. The staphylococci identified were more of the albus type and the streptococci of the viridans and alpha hemolytic type. Streptococci were the most resistant to the antibiotics tested.

TABLE I
PERCENTAGE OF NEGATIVE CULTURES AMONG TEETH IMMERSED IN ANTIBIOTIC SOLUTIONS FOR 2 TO 5 MINUTES

	PER CENT
1. Oxytetracycline 250 mg.*	92.3
2. Penicillin Sodium 75,000 units*	88.1
3. Penicillin Sodium 250,000 units* and Streptomycin 250 mg.*	70.8
4. Oxytetracycline 125 mg.*	66.6
5. Streptomycin 250 mg.*	65.5
6. Penicillin Sodium 100,000 units*	62.6
7. Chlortetracycline 166 mg.*	50.0
8. Neomycin Sulphate 200 mg.*	46.7
9. Penicillin G Procaine	44.4
10. Bacitracin 10,000 units*	42.4
11. Benzathine Penicillin G 300,000 units*	41.7
12. Chloromycetin 33.3 mg.*	37.0
13. Penicillin G Sodium 250,000 units*	33.3
14. Penicillin G Potassium 100,000 units* and Sulphonamides 0.5 Gm.*	16.6

*Concentration per cubic centimeter.

From the foregoing findings it is suggested that Terramycin in a concentration of 250 mg. per cubic centimeter of solution may be effective in operative dentistry and endodontics. In sterilization of cavities with or without exposures and pulpotomies the Terramycin solution can be applied with saturated cotton pellets from 2 to 5 minutes, and in root canal therapy it can be injected or applied with saturated paper points and allowed to remain for a minimum of 4 to 5 minutes before fillings are placed. Further in vivo tests should be undertaken.

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VARIATIONS IN NERVE DISTRIBUTIONS OF THE MAXILLARY TEETH

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CLINICIANS have observed considerable variability in the anesthetic effects of procaine when injected by conduction or infiltration. It was felt that careful dissections of the nerve distribution to the maxillary teeth might add to our knowledge of the cause of such variability. In addition, such dissections might help in the more critical diagnosis of obscure pain symptoms.

A review of the anatomic and clinical literature[1-18] indicates that certain details of the nerve supply of the maxillary teeth are in question and recent investigations have been on a limited number of dissections. Fifty maxillae were decalcified and dissected to show the branches from the maxillary and infraorbital nerves in the belief that some addition could be made to our knowledge of the innervation of the maxillary teeth.

MATERIALS AND METHODS

The maxillae used in this study were secured from the anatomy department of the Northwestern University Medical School. The maxillary nerve remained undissected in the specimens. The specimens were carefully cleaned of soft tissue, preserving the nerves of the pterygopalatine fossa and the sinus membrane. They were then placed in formaldehyde for 30 days to harden the nerves.

Specimens 1 through 4, and 9 through 16, were decalcified with dilute hydrochloric acid. The time required varied with the density of the bone and ranged from 5 to 15 days, with an average of 7 days. These specimens were bleached and dissection was difficult. Specimens 5 through 8 were decalcified with dilute nitric acid for 7 days. The bone was well decalcified but the nerves were macerated and difficult to dissect.

After being made basic by soaking in a solution of lithium carbonate, the balance of the specimens was decalcified electrolytically, using a solution of 10 per cent by volume of 90 per cent formic acid and 8 per cent by volume of 37 per cent hydrochloric acid. The time required varied from 5 to 8 days. Decalcification was complete with minimum damage to the nerves to be dissected. Actual dissection of the nerves to the teeth was not possible in all cases.

RESULTS

Posterior Superior Dental Nerves.—The posterior superior dental nerves were dissected in 47 of the specimens and were represented by 1 branch in 21

The data reported are taken from the thesis presented to the Graduate School of Northwestern University in partial fulfillment of the requirements for the degree of Master of Science in Dentistry, 1951.

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(45 per cent) (Fig. 1); by 2 branches in 14 (30 per cent) (Fig. 2); and by 3 branches in 12 (25 per cent) (Figs. 3 and 4) of the specimens.

In 6 of the multiple nerve groups, 1 nerve appeared to supply only the soft tissue in the region of the maxillary tuberosity and third molar region (Fig. 5). In 19 of the dissections a large branch was observed supplying this area, this branch coming from a major branch to the teeth. This was usually the lowest and most posterior nerve branch. In general it was noted, where only one branch was present, that this branch was larger than the multiple nerve branches.

Some of the posterior branches were found to lie between the bone and the sinus membrane in all or part of their paths (Fig. 6).

Where multiple branches were present, the branch entering the highest foramen usually supplied the most anterior teeth (Figs. 2 and 4).

In 12 of the specimens where a middle branch was present, 2 (17 per cent) of the posterior nerves appeared, at least in part, to supply the premolar region, 3 (25 per cent) appeared to supply the teeth only as far as the first molar, while 7 (58 per cent) joined in a nerve plexus.

In 2 cases (Fig. 1), a branch originated in the pterygopalatine fossa above the posterior branches, ran forward high on the maxilla, then coursed toward the alveolar process in the region of the premolar teeth where it appeared to innervate the region.

In some cases it was possible to trace nerve filaments to the roots of the teeth (Fig. 4).

Middle Superior Dental Nerve.—Of the 50 specimens used in this study only 15 (30 per cent) followed the classical description (Fig. 6). The majority of these middle branches entered into the formation of a nerve plexus which supplied the teeth (Fig. 5).

In the 34 (68 per cent) specimens in which no typical middle dental nerve was demonstrated, 2 specimens had nerves supplying the premolar region. These nerves originated in the pterygopalatine fossa, coming off above the posterior dental nerves and coursing obliquely downward to the premolar teeth (Fig. 1).

In 18 (36 per cent) of the specimens in which the typical middle branch was absent, the anterior dental nerve had secondary branches which descended to the area of the premolar teeth, This is in agreement with Cunningham's[2] statement that the middle superior dental nerve may be separate or may be only a secondary branch of the anterior, but in some instances the posterior superior dental nerve supplies the premolar teeth in the absence of the middle superior dental nerve (Figs. 2 to 4). In these cases the posterior dental nerves either ended at the first molar or formed a dental plexus with the anterior nerve.

In most cases the middle superior dental nerve was found to lie between the sinus membrane and the maxillary bone in at least part of its course.

Anterior Superior Dental Nerve.—The origin, course, and distribution of the anterior superior dental nerve conformed most nearly to the classical

description. The exception was found in the 18 specimens having secondary nerve branches to the premolar region, and in which the middle dental nerves were absent (Figs. 2 and 3).

Fig. 1.

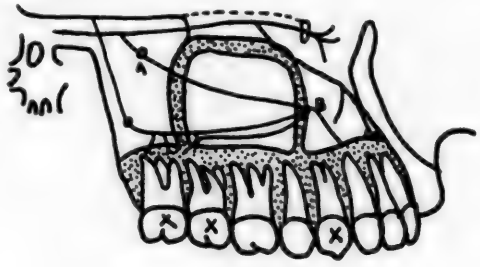

Fig. 2.

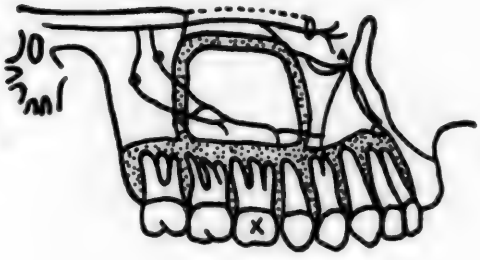

Fig. 3.

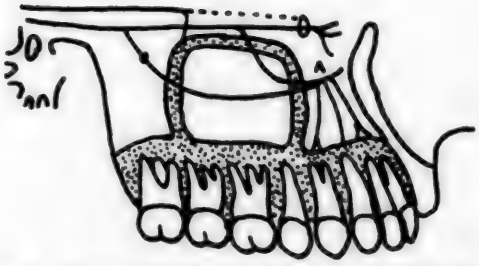

Fig. 1.—Dissection 48. Unusual middle dental nerve originating in pterygopalatine fossa above posterior dental nerve. Point *B* shows an anastomosis between the 3 maxillary dental nerves in what is believed to be a sensory ganglion.

Fig. 2.—Dissection 24. Definite nerve plexus between 2 posterior dental nerves and an anterior dental nerve with secondary branch to premolar area. Middle dental nerve absent. Point *A* is believed to represent a sensory ganglion.

Fig. 3.—Dissection 26. Single posterior dental nerve in a definite plexus with an anterior dental nerve with secondary branch to premolar region. Sensory ganglion believed to be present at point *A* above root of first premolar tooth.

In most cases the origin was from the infraorbital nerve, arising at a variable point in the infraorbital canal. The nerve usually enters the bone a short distance from the infraorbital foramen. It proceeds downward and forward in the bone to a point above the apices of the anterior teeth.

In half of the specimens, the nerve, after entering the bone, passed medially to the nasal process of the maxillae and then proceeded downward to supply the teeth.

In all cases where a middle branch was present, or where the anterior branch was double, definite superior dental nerve plexuses were present.

Superior Dental Plexus.—The presence of a superior dental plexus was definitely established in 24 (48 per cent) of the specimens (Figs. 2 to 4, and 6).

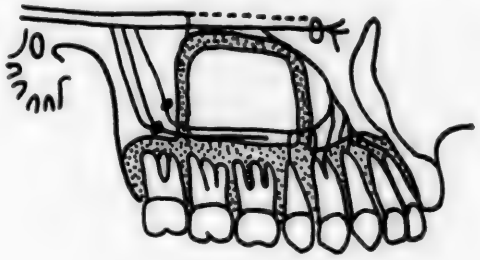

Fig. 4.

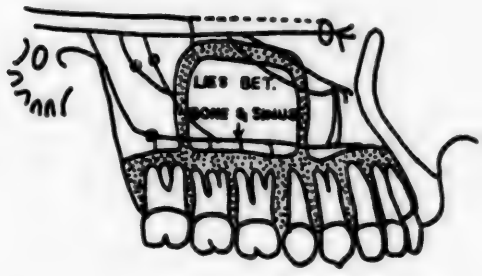

Fig. 5.

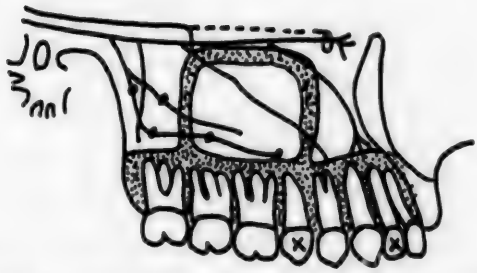

Fig. 6.

Fig. 4.—Dissection 17. Definite nerve plexus composed of 3 posterior dental nerves and an anterior dental nerve with secondary branch to the premolar region. Lines drawn into the teeth indicate that these nerves were actually traced to these teeth.

Fig. 5.—Dissection 50. Definite plexus between 3 posterior dental nerves and the middle and anterior dental nerves.

Fig. 6.—Dissection 1. Generally accepted innervation of the maxillary teeth. Note independent minor branch to tuberosity area and probable anastomoses between the posterior and middle dental nerves. One of the posterior nerves and the middle dental nerve lie between the bone and the maxillary sinus membrane for a part of its course.

In 4 cases (8 per cent) the dental plexus was believed to be present, but its presence was not definitely shown, usually because of poor decalcification. In 11 (22 per cent), the plexus was definitely noted as not being present, while in yet another 11 (22 per cent) the existence of the dental plexus was not determined.

The presence of a superior dental plexus appears to be favored by multiple posterior branches and the presence of a middle branch or an anterior branch with multiple main branches.

In 3 dissections (Figs. 1 to 3), nerve arrangements were noted that appeared to be sensory ganglions. Both of these were associated with nerve plexuses. One of these gangliforms was found between the posterior superior dental and the middle superior dental nerves in one case. This conforms to the ganglion of Valentin, of which Robinson[13] stated that when present it is found over the second upper premolar. In another case the ganglion was found between the secondary branches of the anterior dental nerve and the posterior dental nerve, and the third case found it between the secondary branches of the anterior dental nerves. This latter ganglion, when found between the anterior and middle dental nerves, has been called the ganglion of Bochdalek.

DISCUSSION

The composite views of the anatomists have been verified by this group of dissections.

The posterior superior dental nerve was found to be a single nerve in 45 per cent of these dissections, as indicated by the earlier anatomists,[5] while 55 per cent of the specimens revealed multiple nerve branches, which agrees with the later investigators.[2, 8, 10, 12, 14, 15, 18]

The greatest variation appears in the formation of the middle superior dental nerve, with only 30 per cent of the specimens following the usual description. It has been stated[9, 18] that the middle nerve may be absent, or may be a branch of the anterior superior dental nerve,[2, 18] which was verified in 36 per cent of the cases in this study.

The anterior superior dental nerve was found to be present in all of the specimens of this study. In many cases it was the largest of the nerves. The posterior dental nerves were traced to the premolar region in 26 per cent of the specimens. In 12 per cent of the cases the anterior dental nerve was traced to the premolar region. In 48 per cent of the cases, the teeth were found to be innervated from a dental plexus which was the result of an anastomosis between the dental nerves.

The above innervation of the upper premolar teeth has been partially substantiated clinically by the writer. In 15 cases where the first or second upper premolar teeth were tender to percussion and the patient complained of acute symptoms, the teeth were extracted, painlessly, without the usual buccal infiltration of procaine. It was found that either an infraorbital or zygomatic block injection, or both, gave complete anesthesia to the teeth. The infraorbital injection gave complete anesthesia to the first premolar tooth more frequently than it did to the second premolar. This clinical finding agrees in part with Scharlau's[16] anesthetic zones.

CONCLUSIONS

1. Many variations were found in the origin, course, and distribution of the maxillary nerves to the teeth.

2. In 48 per cent of the cases the maxillary teeth were supplied by branches from a dental plexus.

3. The posterior dental nerves may consist of 1, 2, or 3 branches.

4. The middle dental nerve is inconstant, being present in 17 (34 per cent) of the specimens.

5. The anterior dental nerve was always present and, in many cases when the middle dental nerve was absent, this nerve had a secondary branch which supplied the premolar region.

6. The posterior dental nerve was traced to the premolar region in 13 (26 per cent) of the dissections.

7. The anterior dental nerve was traced to the premolar region in 6 (12 per cent) of the dissections, where only single branches were present. In 18 (24 per cent) of the specimens where the middle dental nerve was absent a secondary branch of the anterior supplied the premolar region.

8. Vague maxillary pain in the premolar region may be explained by maxillary sinus infection which could cause pressure or irritation to the superior dental nerve.

9. Lack of uniformity in procaine anesthesia by conduction or infiltration may be explained by variations in the anatomic distribution of the maxillary nerve.

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THE EFFECT OF DESICCATED THYROID, PROPYLTHIOURACIL, TESTOSTERONE, AND FLUORINE ON THE SUBMAXILLARY GLANDS OF THE RAT

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WITH THE TECHNICAL ASSISTANCE OF PATRICIA G. CLARK

A RELATIONSHIP between the thyroid gland and the salivary glands has been suggested by occasional reports appearing in the literature during the last few years. Previous to this, a relationship between other endocrine organs and the salivary glands was recognized by the fact that gonadectomy or the administration of various sex hormones profoundly alters the structure of the salivary glands in experimental animals.[1-4] The function of the salivary glands, as judged by the determination of the amylolytic activity of the saliva, has been shown to be altered by castration in mice,[5] while a possible functional relationship between the hypophysis and the salivary glands also has been demonstrated in mice and rats.[6-8]

That the thyroid gland is directly related to the structure, function, and metabolism of the salivary glands has been suggested by the work of Grad and Leblond,[9] who have shown alterations in the structure of the rat submaxillary gland concomitant with alterations in function of the thyroid gland. In addition, testosterone was shown to possess a synergistic effect with the thyroid hormone on the salivary gland.[9, 22] Burris, Bogart, and Krueger[10] have reported an increase in the weight and activity of the thyroid gland of cattle administered testosterone.

Raynaud[11] has shown that the direct injection of testosterone into the submaxillary glands of mice resulted in a marked hypertrophy of the tubules and he interpreted this as evidence of a direct action of this androgen on the gland. Arvy and Gabe[12] produced atrophy of the submaxillary gland tubules in mice by thyroidectomy and found that the atrophy disappeared upon administration of thyroxine to the thyroidectomized animals. Arvy, Debray, and Gabe[13] further showed that thiourea produced tubular atrophy of the submaxillary glands similar to that produced by thyroidectomy. Gabe[14] has also reported that the administration of thyroxine to hypophysectomized rats caused disappearance of the atrophy of the submaxillary gland tubules which follows hypophysectomy. An increase in the nuclear volume of tubular cells of mice submaxillary glands following the administration of testosterone has been reported by Valeri,[15] and he believed this indicates increased secretory activity of the tubular cells.

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Fawcett and Kirkwood[16] have proposed that one of the important functions of the salivary glands is to control the level of thyroxine in the blood by deiodinating thyroxine and recycling the iodide ion to the thyroid gland via the saliva and gastrointestinal tract. Their work clearly indicates that the level of thyroxine in the blood is controlled not only by the rate of synthesis of the thyroid hormone but also by the rate of degradation in the salivary glands. Therefore, the salivary glands function as "reverse thyroid" organs, and have an extremely important role in the extra thyroidal metabolism or organic iodide in the body. A report by Albright, Larson, and Deiss[17] demonstrating hypertrophy of the salivary glands following the administration of triiodothyronine for myxedema in the human may aid in the confirmation of this hypothesis. Previously, Reiss, Halkerston, Halkerston, and Badrick,[18] on studies of radioactive iodine uptake and excretion in humans, have shown that the saliva of some patients contained up to 600 times more I^{131} per 100 mg. wet weight than the blood and stated, "It appears that this concentrating activity of the salivary glands has some relation to the state of the thyroid function."

A portion of the studies to be reported here was prompted by an interesting theory suggested by Rathje[19] which bears directly on the possible mechanism of action of fluoride in reducing dental caries through a salivary gland-thyroid relationship. Since it is a well-established fact that the function of an organ may be reflected in its histologic structure and, more specifically, that since the alteration in function of the salivary glands, as judged by their histologic structure, may alter the incidence of dental caries, the implication would be obvious if the thyroid gland does influence the salivary glands: thyroid gland function may be of prime importance in determining dental caries activity.

EXPERIMENTAL METHODS

This study was divided into 2 series: in the first series, 215 weanling albino rats (Sprague-Dawley strain), of which 161 survived the experimental period of 145 days, were divided into 6 groups. Group I were controls and received no treatment; Group II received desiccated thyroid in the diet, increasing from 10 to 60 mg. per day depending on the age and growth response of the animal; Group III received propylthiouracil in the diet at a concentration of 100 mg. per 100 Gm. of food; Group IV received 20 ppm of fluoride (as NaF) in the drinking water; Group V received the same amount of desiccated thyroid as Group II and, in addition, received a similar amount of fluorine as the animals in Group IV; Group VI received the propylthiouracil diet plus the same amount of fluoride as Group IV. All of the animals were housed in pairs in wire bottom cages and received a stock corn cariogenic diet.[20] Food and water were available ad libitum.

The second series consisted of 240 weanling Sprague-Dawley rats of which 195 animals survived the 126-day experimental period. The animals were divided into 6 groups and received the following supplements: Group I were controls and received no treatment; Group II received in increasing amounts 1 to 20 mg. daily of desiccated thyroid in the diet, depending on the age and general appearance of the animals; Group III received propylthiouracil in the

diet at a concentration of 100 mg. per 100 Gm. of food; Group IV received 1.25 mg. of testosterone* per week injected intramuscularly; Group V received the same amount of thyroid as Group II, while Group VI received the same amount of thiouracil as Group III. In addition, both Groups V and VI received the same amount of testosterone as Group IV. The cariogenic diet and housing conditions were identical to those described in the former series.

At the termination of the studies, the animals were sacrificed by chloroform inhalation and the salivary glands removed for fixation in Helly's fluid. Sections of these glands were stained with hematoxylin and eosin as well as with Mallory's one-step aniline blue since the latter has been found to give better visualization of the structures of interest in the submaxillary glands. Since no marked changes were noted in the parotid or sublingual glands, the present discussion pertains only to the submaxillary glands.

The particular structure which appeared to exhibit structural alteration under the conditions of these studies was the "serous tubule" or "granular tubule" which, anatomically, appears to lie between the acinus and the intralobular excretory duct. Normally, these tubules are small and sparse in the weanling animal but appear larger and in greater numbers as the animal ages. The functions of these granules are not known although they appear to be discharged into the excretory tubules in the same form as they appear intracellularly. The relative presence or absence of these granules could markedly affect the composition or consistency of the salivary secretion. The method used to determine differences in these tubules between the various experimental groups is the measurement of their diameter by means of a micrometer eyepiece.

RESULTS

In the first series, the differences between control and thyroid-treated animals were immediately apparent (Table I). The granular tubules of the latter group were significantly larger than those of the controls and, while the measurements do not indicate this, the tubules were choked with granules (Fig. 1). The thiouracil-treated animals similarly exhibited marked differences from the controls, showing a marked diminution in size of the tubules with an almost complete disappearance of granules. It is interesting that the fluoride-treated animals showed no differences when compared to the controls. The simultaneous administration of thyroid and fluoride, or thiouracil and fluoride produce no consistent changes of a magnitude greater or lesser than those seen in animals treated only with thyroid and thiouracil, respectively. Thus, no evidence could be found, based on the histologic appearance of the submaxillary glands, that fluoride in any way directly altered the function of the salivary glands. However, it is well recognized that the function of an organ may be altered without histologic evidence of such alteration. Such a condition is still a possibility with regard to fluoride and the salivary glands.

In the second series, testosterone was utilized to determine whether a synergism with the thyroid hormone exists. As in the previous study, it was

*The authors wish to thank Schering Corp., Bloomfield, New Jersey, for supplying the testosterone (Oreton-F) used in these studies.

found that desiccated thyroid produced a marked increase in the size of the tubules with the accumulation of large numbers of granules within the cells, while thiouracil resulted in a pronounced decrease in the size of the tubules

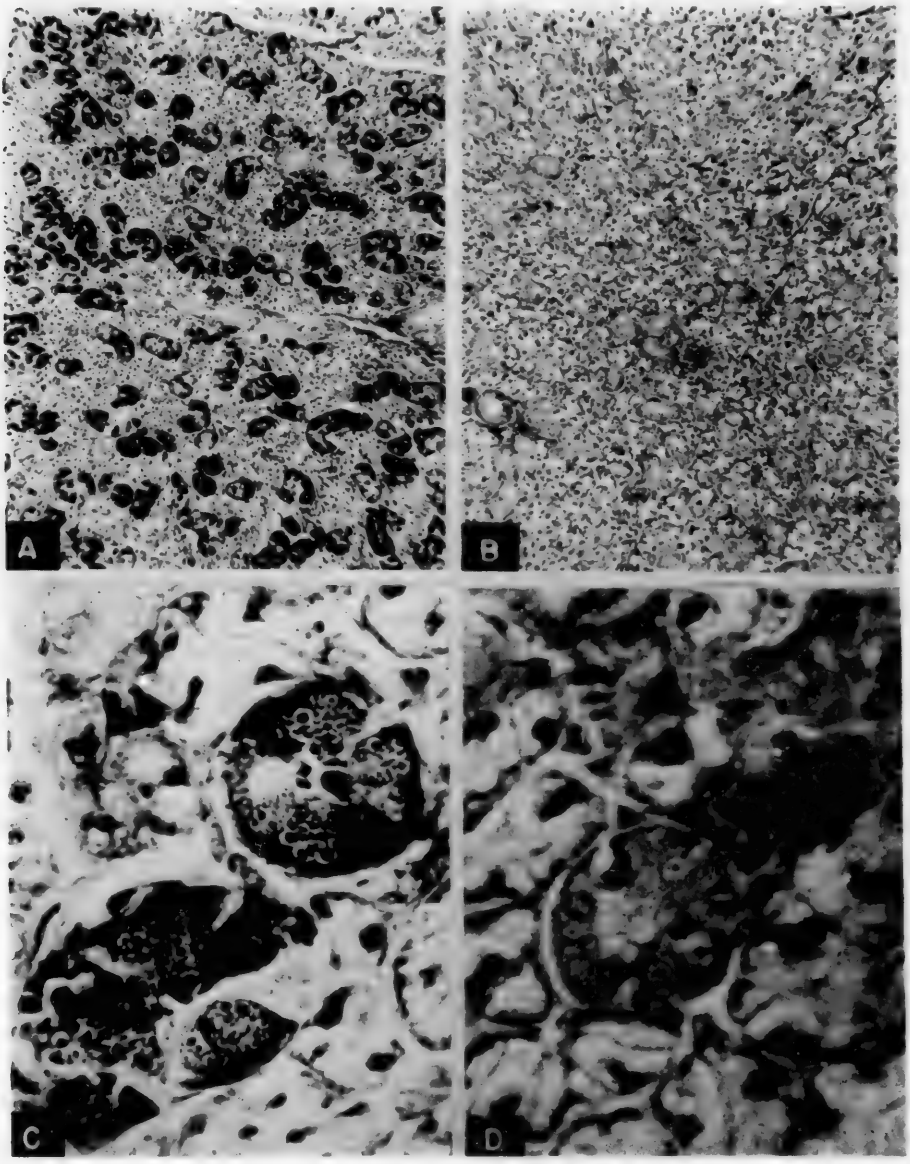

Fig. 1.—*A*, Submaxillary salivary gland of rat receiving desiccated thyroid shows large, granule-choked tubules. *B*, Submaxillary salivary gland of rat receiving propylthiouracil shows nearly complete absence of granules and diminution in size of the tubules. *C*, High-power photomicrograph of a single tubule in thyroid-treated rat shows accumulation of granules. *D*, High-power photomicrograph of a single tubule in thiouracil-treated rat shows loss of granules.

TABLE I

EFFECT OF DESICCATED THYROID, PROPYLTHIOURACIL, AND FLUORINE ON THE SIZE OF GRANULAR TUBULES OF THE SUBMAXILLARY GLAND OF THE RAT

			MEAN DIAMETER OF TUBULES (MICRA)	STANDARD DEVIATION	PROBABILITY OF SIGNIFICANCE†			
					GROUPS	DIFFERENCE	d AT .05 LEVEL	d AT .01 LEVEL
MALES	(1)	Control	39.3*	2.2	1 vs. 2	35.6	10.42	12.55
	(2)	Thyroid	47.1	1.3	1 vs. 3	16.7	10.42	12.55
	(3)	Thiouracil	35.6	1.1	2 vs. 3	52.3	10.42	12.55
	(4)	Fluorine	41.4	1.5	1 vs. 4	9.5	10.42	12.55
	(5)	Thyroid-fluorine	47.3	2.3	2 vs. 5	2.9	12.23	14.42
	(6)	Thiouracil-fluorine	33.7	1.4	3 vs. 6	9.0	10.42	12.55
FEMALES	(7)	Control	40.0	1.2	7 vs. 8	23.4	15.31	18.45
	(8)	Thyroid	45.2	1.3	7 vs. 9	43.7	13.94	16.80
	(9)	Thiouracil	30.4	1.3	8 vs. 9	67.1	15.31	18.45
	(10)	Fluorine	41.2	2.1	7 vs. 10	6.0	13.94	16.80
	(11)	Thyroid-fluorine	45.5	2.8	8 vs. 11	1.7	15.31	18.45
	(12)	Thiouracil-fluorine	34.0	1.8	9 vs. 12	16.3	13.94	16.80

*All measurements are based on 10 animals from each group selected at random, with 100 measurements made in each group.

†In the method of analysis used in this study, if the difference is equal to or greater than the given d – value, it is considered significant at the probability level associated with the d – value. It should be noted that the difference is not the mathematical difference of the means between the various groups but is a difference derived in the analysis.

For method see: Scheffe, H.: A Method for Judging All Contrasts in the Analysis of Variance, Biometrika 40: 87, 1953.

TABLE II

EFFECT OF DESICCATED THYROID, PROPYLTHIOURACIL, AND TESTOSTERONE ON THE SIZE OF GRANULAR TUBULES OF THE SUBMAXILLARY GLAND OF THE RAT

			MEAN DIAMETER OF TUBULES (MICRA)	STANDARD DEVIATION	PROBABILITY OF SIGNIFICANCE†			
					GROUPS	DIFFERENCE	d AT .05 LEVEL	d AT .01 LEVEL
MALES	(1)	Control	44.0*	2.8	1 vs. 2	28.3	14.25	17.15
	(2)	Thyroid	50.2	1.7	1 vs. 3	51.1	14.25	17.15
	(3)	Thiouracil	32.8	1.7	2 vs. 3	79.4	14.25	17.15
	(4)	Testosterone	46.3	2.9	1 vs. 4	11.2	14.25	17.15
	(5)	Thyroid-testosterone	53.2	1.7	1 vs. 5	42.0	14.70	17.69
	(6)	Thiouracil-testosterone	34.2	1.1	1 vs. 6	44.8	14.25	17.15
					4 vs. 5	30.8	14.70	17.69
					4 vs. 6	56.0	14.25	17.15
					2 vs. 5	13.7	14.70	17.69
					3 vs. 6	6.3	14.25	17.15
FEMALES	(7)	Control	39.5	2.0	7 vs. 8	28.8	15.51	18.66
	(8)	Thyroid	45.8	3.7	7 vs. 9	30.4	15.51	18.66
	(9)	Thiouracil	32.8	1.9	8 vs. 9	59.2	15.51	18.66
	(10)	Testosterone	44.6	2.2	7 vs. 10	23.3	15.51	18.66
	(11)	Thyroid-testosterone	49.7	2.6	7 vs. 11	46.6	15.51	18.66
	(12)	Thiouracil-testosterone	33.5	1.6	7 vs. 12	27.2	15.51	18.66
					10 vs. 11	23.4	15.51	18.66
					10 vs. 12	50.4	15.51	18.66
					8 vs. 11	17.8	15.51	18.66
					9 vs. 12	3.2	15.51	18.66

*All measurements are based on 10 animals from each group selected at random, with 100 measurements made in each group.

†In the method of analysis used in this study, if the difference is equal to or greater than the given d – value, it is considered significant at the probability level associated with the d – value. It should be noted that the difference is not the mathematical difference of the means between the various groups but is a difference derived in the analysis.

For method see: Scheffe, H.: A Method for Judging All Contrasts in the Analysis of Variance, Biometrika 40: 87, 1953.

with a concomitant loss of granules (Table II). Testosterone alone in the males was found to cause a slight but statistically insignificant increase in the size of the tubules in the males. This is in contradistinction to a study previously reported[4] in which a significant increase in size was noted. However, in the females in this present study, testosterone did produce a definite significant increase in tubular diameter. Of interest was the fact that desiccated thyroid and testosterone administered simultaneously produced an enlargement of tubules considerably greater than that produced by either testosterone or thyroid alone, but this was not found to be statistically significant. The reason for this may be that the tubules were enlarged to the maximum level and that while a true increase was present, this could not be directly evaluated by the present methods of detection. Testosterone administered with thiouracil caused alterations of no greater magnitude than those produced by thiouracil alone.

DISCUSSION

The studies reported here suggest that an intimate relationship exists between the rat thyroid gland and submaxillary gland. It is quite possible that such alterations in structure may result in changes in the salivary secretion, although direct evidence for this is not available at the present time. This is of prime dental interest since changes in the nature of the saliva might easily produce changes in the incidence of dental caries and periodontal disease. Experimental dental caries studies were carried out on the animals described above and have been reported elsewhere.[21, 22] In these studies it was found that thiouracil caused a marked increase in dental caries incidence while thyroid produced a marked reduction. Further, thyroid and testosterone produced a much greater reduction in dental caries than either acting alone. It may be seen, therefore, that experimental rat dental caries incidence closely parallels the pattern or structure of salivary glands. A similar relationship has been reported in hypophysectomized animals.[8]

It appears that many procedures, including gonadectomy, administration of various male and female sex hormones, and hypophysectomy, can produce alterations in the structure of the submaxillary glands. In addition, extremely severe changes have been noted in animals receiving propylthiouracil or desiccated thyroid, particularly when the latter is combined with testosterone. Clarification of this phenomenon, particularly as to whether this represents a functional change in the salivary glands with the concomitant alteration in the incidence of dental caries, awaits further study.

SUMMARY AND CONCLUSIONS

Two studies have been conducted to evaluate the effect of thyroid function on the structure of the rat submaxillary salivary glands. In the first series, the administration of desiccated thyroid and of propylthiouracil resulted in marked variations in the histologic pattern of this gland, while fluoride, either alone or in conjunction with desiccated thyroid and propylthiouracil, produced no additional significant changes.

In the second series of observations, desiccated thyroid and propylthiouracil were administered alone as well as testosterone alone and in conjunction with the desiccated thyroid and propylthiouracil. The two latter compounds resulted in alterations in the submaxillary gland identical with those noted in the first study. Testosterone alone produced no significant changes in the male, but the changes in the female were of a significant magnitude. Testosterone in combination with propylthiouracil produced no additional changes from those seen in animals treated with the propylthiouracil alone. The concomitant administration of testosterone and desiccated thyroid produced greater changes than when either of the two was administered alone. However, these former alterations were found not to be statistically significant.

It is suggested that an intimate relationship exists between thyroid gland function, the salivary glands, and experimental dental caries in the rat.

We wish to thank Dr. Arthur W. Radike and Mr. Robert Lenhoff for the statistical analysis of these data.

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THE INFLUENCE OF DIETS CONTAINING FATS WITH HIGHLY UNSATURATED FATTY ACIDS UPON THE MAXILLARY INCISOR TEETH OF RATS

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IT WAS reported by Granados and Dam[6] and by Granados, Mason, and Dam[7, 8] that diets containing fats with a high content of highly unsaturated fatty acids caused degeneration of the enamel organ and depigmentation of the incisor teeth of rats, these changes being prevented by the administration of α-tocopherol. Irving and Budtz-Olsen[14] repeated these experiments and found that the results obtained depended on the type of liver oil used as a source of the highly unsaturated fatty acids. Using cod-liver oil, certain changes were obtained similar to those described by Irving[11] in vitamin E deficiency, and these changes were avertable by α-tocopherol dosage. If hake-liver oil was used at the same level in the diet (10 or 20 per cent), histologic changes in the enamel organ were obtained which differed in many respects from those obtained with the cod-liver oil diet and which were not prevented by α-tocopherol administration. It was suggested that this was due to the high content of vitamin A in hake-liver oil. The present paper describes these histologic changes, using both cod-liver oil and hake-liver oil.

EXPERIMENTAL

Diets.—Diets were based on that of Gillman, Gilbert, Gillman, and Spence[5] and consisted of potato starch, dried brewers' yeast, and fish-liver oil. In all diets the level of yeast was 20 per cent; the fish-liver oil was at different levels, the balance being made up from potato starch. A thick paste was made of the starch in boiling water. After this had cooled, the other ingredients were thoroughly stirred in. Using cod-liver oil, the levels of oil in the diet were 20, 10, or 2.5 per cent. As the changes observed in the teeth were the same with these 3 levels, the diets using cod-liver oil will be collectively referred to as Diet 1. With hake-liver oil 20 or 10 per cent levels were used. Since again the dental results were the same with both levels, these 2 diets will be referred to collectively as Diet 2. Control animals were given 3 mg. of α-tocopherol acetate (Ephynal, Roche) daily in 3 Gm. of freshly made diet.

Rats.—Albino rats of the Wistar Institute strain were employed. Both sexes were used indiscriminately, as it was found that similar results were got with each. The rats were placed on the diets when they weighed about 50 grams. Forty-two rats were used on Diet 1, half on the diet alone, and half getting α-tocopherol in addition. Forty rats were placed on Diet 2, 28 being on the diet alone, and 12 getting α-tocopherol supplements. The number and days of killing of all groups are given in Table I.

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TABLE I
TREATMENT OF RATS AND NUMBER KILLED ON VARIOUS DAYS AFTER THE EXPERIMENT BEGAN

DIET	SOURCE OF HIGHLY UNSATURATED FATTY ACIDS	α-TOCOPHEROL	DAYS						
			10	15	20	30	40	50	60
1	Cod-liver oil	None	–	–	3	6	9	2	1
1	Cod-liver oil	3 mg. daily	–	–	3	6	9	2	1
2	Hake-liver oil	None	3	1	5	15	4	–	–
2	Hake-liver oil	3 mg. daily	–	–	3	8	1	–	–

After death, the color of the upper incisor teeth was noted; blood was taken for the dialuric acid hemolysis test (György and Rose[9]); and the skulls were decalcified and central longitudinal sections of the upper incisor teeth were stained with hematoxylin and eosin.

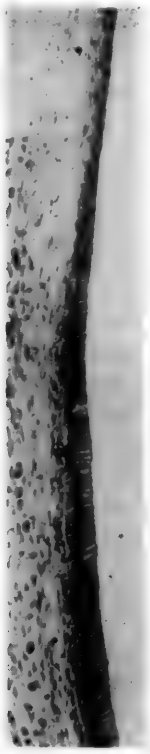
RESULTS

Diet 1 (Containing Cod-Liver Oil).—The enamel organs of all rats on this diet without α-tocopherol were affected. The most striking change was the disappearance of the enamel organ and its replacement by fibrous tissue covered by a very thin layer of flattened cells and containing many macrophages. This change might be gradual as one progressed from the formative to the incisal end of the tooth, the epithelial papillae becoming flatter, then the ameloblasts becoming reduced in height, and the whole enamel organ becoming narrower by degrees (Fig. 1); the outer enamel epithelium then appeared as a condensed layer against the flattened ameloblasts and often two distinct layers of nuclei were seen; or else the whole enamel organ might suddenly become thinner and disappear in a distance of 10 to 15 ameloblasts (Fig. 2).

Whether the change was sudden or gradual, the end result was the same, the site of the enamel organ being occupied by fibrous tissue (Fig. 3). Qualitatively the change was the same in all rats irrespective of the time on the experiment, or cod-liver oil content of the diet; however, with the passage of time, the site of the disappearance of the enamel organ moved more toward the formative end. Thus, after 20 days it was near the junction of the formative two-thirds and the incisal one-third, while after 60 days it was at the junction of the formative one-third and the incisal two-thirds. In no cases, however, did it occur on the formative side of the granular ameloblasts (those containing translucent pigment granules). It was either in front of the most incisal of these, and sometimes considerably anterior, or else the degeneration might occur in the last and most incisal of these cells.

In a few animals the ameloblasts reappeared on the incisal side of the atrophied enamel organ on the edge of the fibrous tissue, often accompanied by epithelial papillae. Here the ameloblasts and epithelial papillae were narrower than normal (Fig. 4). Three of the animals showed small cysts of the papillary layer like those described by Pindborg[17, 18] as occurring in vitamin E deficiency. More incisal of these, the papillary layer was again normal. These cysts were not common, and the piling up of ameloblasts to form cysts, also described by Pindborg, was not seen. In one animal the ameloblast layer disappeared, but the papillary layer persisted almost to the crest of the alveolar bone.

A striking feature of the fibrous tissue which replaced the enamel organ was the number of large macrophages with eccentric nuclei embedded in it (Fig. 3). These have been described by Irving[13] and contained refractile orange granules with histochemical properties similar to those of the granules in the granular ameloblasts and to those of the yellow pigment on the enamel. These cells occurred under the enamel organ where it was very degenerate and in the fibrous tissue which replaced the enamel organ.



Figs. 1-6.—All are photomicrographs in the middle third of the enamel organ of the upper incisor tooth.

Fig. 1.—Rat No. 2260, on Diet 1 for 40 days, showing gradual atrophy and final disappearance of all layers, which are replaced by fibrous tissue. (×130.)

None of the histologic changes described above were seen in the animals receiving α-tocopherol. In these the enamel organ was entirely normal. With the exception of one 20-day rat, the upper incisor teeth of all the rats on the α-tocopherol-free diet were white, either entirely, or else the pigment was found only on the tip of the tooth, leaving a white area adjacent to the gingival margin. The teeth of all the control animals were of the normal color. The dialuric acid hemolysis test was positive in all the rats on the α-tocopherol-free diet and negative in all dosed with α-tocopherol.

Diet 2 (Containing Hake-Liver Oil).—Many sections showed changes identical with those in rats on the cod-liver oil diet. A considerable number however showed differences which are listed as follows: (1) In the earliest stage of the lesion, the enamel organ changed to undifferentiated cells mixed with macrophages for a short distance or else became a small cyst filled with macrophages and cell debris. Normal enamel organ occurred again on the incisal side. This change was seen very far forward in the enamel organ near the crest of the alveolar bone. (2) Vacuolated ameloblasts were sometimes seen among the normal ameloblasts on the formative side of the atrophy. The last

Fig. 2.

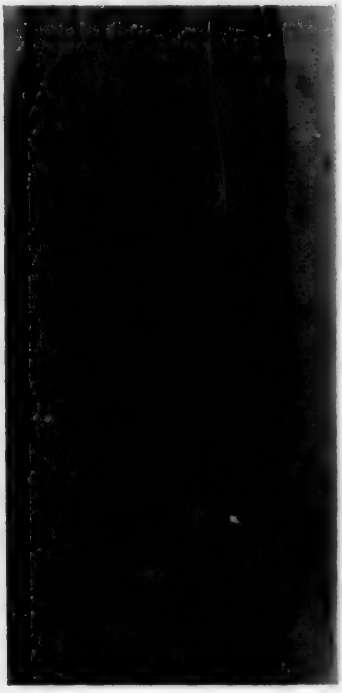


Fig. 3.

Fig. 2.—Rat No. 2154, on Diet 2 for 30 days, showing sudden atrophy of all layers of the enamel organ. (×260.)

Fig. 3.—Rat No. 2238, on Diet 1 for 60 days, showing replacement of all enamel organ by fibrous tissue containing large macrophages. (×400.)

50 or so ameloblasts before the final atrophy often stained much paler with hematoxylin. (3) The atrophied enamel organ was not succeeded at once by fibrous tissue, but changed into a mixture of necrotic cells and macrophages. (4) The atrophy was more extreme. The whole enamel organ suddenly collapsed, the outer enamel epithelium became condensed and the ameloblasts disappeared, leaving a cyst containing macrophages and coagulated protein, lined on the tooth side by a very fine line of flattened cells and on the outer side

by compressed outer enamel epithelium, undifferentiated cells, or fibrous tissue (Fig. 5). Ghosts or debris of ameloblasts were sometimes seen inside the cyst (Fig. 6). The enamel organ might recover anterior of the cyst and then disappear, being replaced by fibrous tissue, or else another cyst might form. The macrophages in the cyst phagocyted the eosinophil granules of the degenerated granular ameloblasts or else the granules lay loose between the degenerated cells. (5) In several sections mitotic figures were observed in the fibroblasts of the tissue between the enamel organ and the alveolar bone. These mitoses were

Fig. 4.—Rat No. 2256, on Diet 1 for 30 days, showing an area of atrophy of the enamel organ with normal enamel organ on either side. (×130.)

invariably under the enamel organ lesions, and usually at the formative end of them. While mitoses are not uncommon in fibroblasts in this area, there appeared to be more mitoses in these abnormal sections than usual.

Histologic changes were seen in all rats on the diet without α-tocopherol with one exception (20 days) from 15 days on. Many teeth were white at 30 days and all but one were white at 40 days. No protection was given by α-tocopherol at the dosage used; of those on the diet and receiving the vitamin,

all but 2 had degenerative changes in the enamel organ at and after 20 days, and the teeth were white by 40 days. The dialuric acid hemolysis test was uniformly positive in all rats on the diet without α-tocopherol and negative in all rats dosed with the vitamin.

Fig. 5.

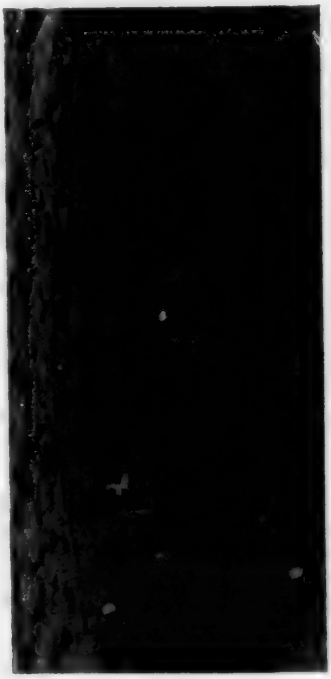


Fig. 6.

Fig. 5.—Rat No. 2104, on Diet 2 for 20 days, showing the sudden collapse and disappearance of the ameloblasts, their place being taken by a cyst containing debris, macrophages, etc. The outer enamel epithelium is compressed. (×260.)

Fig. 6.—Rat No. 2123, on Diet 2 for 15 days. Change similar to that in Fig. 5, but many ameloblasts have persisted in a degenerated form. (×220.)

DISCUSSION

The results show that the curative action of α-tocopherol is governed by the type of oil used as a source of highly unsaturated fatty acids. There is no doubt that the changes in the enamel organ and tooth color, using the diet with cod-liver oil, are both due to α-tocopherol deficiency since they can be averted by the administration of this vitamin. The changes are identical with those originally described by Irving[11] and probably the same as those reported by Granados, Mason, and Dam,[7, 8] though these writers described their findings briefly and verbally with no photomicrographs. Pindborg[17, 18] has described changes in the enamel organ of the rat's incisor tooth, also avertable by α-tocopherol dosage, but these differed in certain ways from those noted in the present

study. Thus the premature atrophy of the papillary layer which he emphasized was not conspicuous in the present results, though the epithelial papillary layer often became reduced in width before the ameloblasts; the edema at the base of the ameloblasts was not seen at all. Small cysts were seen in 3 animals in the papillary layer, but they were not caused by the piling up of ameloblasts as described by Pindborg. He did not comment on the fibrous tissue which replaced the enamel organ or on the presence of macrophages which were such a marked feature of the present experiments.

The differences in the histologic appearances described by Pindborg and the present writer raise some interesting points, since, in both cases, the changes were avertable by α-tocopherol dosage. Furthermore, in the present experiments the changes, both in depigmentation and the enamel organ degeneration, were seen sooner than in the case of Pindborg's rats. These differences must be ascribed to the diets used. With one exception, Pindborg's diets were low in fats. Although Granados, Mason, and Dam,[7, 8] have stressed the importance of fats containing highly unsaturated fatty acids in the diet for producing white teeth and histologic changes in the enamel organ, other writers have obtained white teeth and changes in the enamel organ using diets containing very little fat, but in the latter cases this change took longer to occur. Thus it appears that the lesions caused by vitamin E deficiency depend on the diet used and are probably related to the speed of onset, which is greater with high-fat diets. The changes described by the present writer were acute changes and those by Pindborg were of a more chronic nature. These chronic changes presumably ultimately resemble the acute ones, for most of the enamel organ was replaced by fibrous tissue in rats for 167 days on a diet deficient in vitamin E and containing 4.5 per cent fat and less than 1 per cent of cod-liver oil (Irving[11]).

Since the changes produced by the hake-liver oil diet were not prevented by α-tocopherol, it might be argued that these conditions were due to the toxic action of some constituent in the hake oil and had no relation to vitamin E deficiency. The fact that the lesions, both in their site in the enamel organ and in their appearance, resembled in many cases those prevented by α-tocopherol in rats on the cod-liver oil diet makes it seem more probable that hake oil, or some constituent of it, caused a conditioned α-tocopherol deficiency of much greater severity than that seen with cod-liver oil. This would explain the severity of the lesions and possibly the inability of α-tocopherol in the dosage used to prevent the condition. The fact that the dialuric acid hemolysis test was negative in the animals receiving α-tocopherol shows that this antagonistic action of hake-liver oil to α-tocopherol with respect to the teeth was not universal throughout the whole economy of the body. Irving and Budtz-Olsen[14] have suggested that this action of hake-liver oil might be due to its high content of vitamin A. Irving and Budtz-Olsen[14] found that extra protein added to Diet 1 protected the enamel organ but depigmentation of the enamel still occurred, the dialuric acid hemolysis test being positive, and concluded that α-tocopherol must protect the color of the teeth both by acting in a biochemical way and also by maintaining the structure of the enamel organ. Furthermore, degeneration of the enamel organ and depigmentation of the tooth do not always coincide

(Irving,[11] Pindborg[17]), degeneration occurring but the tooth still possessing a normal color. Irving[13] brought forward evidence that, under his experimental conditions, when enamel organ degeneration occurred, depigmentation was due to the removal of pigment by macrophages. It appears that the process may be rather more complicated under other experimental conditions than this simple explanation would allow.

Weinmann[21] has divided the enamel organ of the rat's incisor into 6 sectors, which form a convenient way of localizing the various events in amelogenesis. From the point of view of the present argument the important sectors are IV, where the ameloblasts become tall and lay down enamel matrix, and sectors V and VI. Sector V is concerned with enamel calcification and VI contains the granular ameloblasts which lay the pigment on the enamel surface.

Different parts of the enamel organ are damaged by various experimental procedures, with corresponding effects upon the erupted tooth. Fluorine appears to affect all of these 3 sectors since the tooth after eruption may be hypoplastic, poorly calcified, and depigmented (Schour and Smith[19]). Butcher[2] has shown that F interferes with pigment formation by the granular ameloblasts in sector VI.

Most other experimental procedures affect one part of the enamel organ only. Thus, lack of fat-soluble vitamins (Mellanby[16]), rickets (Becks and Ryder[1]), magnesium deficiency (Irving[10]), strontium injections (Weinmann[21]), inoculation of tubercle bacilli (Kreshover[15]), vitamin C deficiency (Wassermann[20]), administration of thiouracil (English[4]) and the reimposition of rickets after healing (Irving[12]) all affect the tall ameloblasts of sector IV, causing hypoplasia and often hypocalcification. Chase,[3] by dietary and traumatic means, damaged the ameloblasts of sector IV and produced hypoplasia and hypocalcification.

All these procedures affect the ameloblasts at the formative and calcifying stage of amelogenesis. In the present experiments these ameloblasts were quite unaffected and the teeth did not show hypoplasia or malcalcification. The ameloblasts in sector VI, and usually well forward in this area, were the ones affected here. It must be concluded that different parts of the enamel organ vary in their sensitivities to lack of various nutrients or to the toxic action of various agents, one part being affected and another quite unharmed. The anterior part of the enamel organ is sensitive to α-tocopherol deficiency and, if depigmentation can be taken as a criterion, to vitamin A deficiency as well. The enamel organ of the continuously erupting tooth is not a single tissue, biochemically speaking but, with its changing chemical activities, likewise changes in its sensitivities and requirements from area to area.

SUMMARY

1. Young rats were put on vitamin E-free diets containing either cod-liver oil or hake-liver oil, and were sacrificed at intervals. The enamel organs of the upper incisor teeth were examined histologically and the color of the teeth noted.

2. Using a diet containing cod-liver oil, histologic changes like those described by Irving[11] were obtained and the teeth became white, both changes

being averted by α-tocopherol dosage. The changes differed in some respects from those described by Pindborg[17, 18] and it is suggested that the animals in his experiments were suffering from a more chronic form of vitamin E deficiency.

3. The histologic changes using hake-liver oil were considerably more extreme than those caused by the cod-liver oil diet, and neither the microscopic changes nor the whitening of the teeth was prevented by α-tocopherol dosage.

4. With both types of diet, the affected ameloblasts were on the incisal side of the granular ameloblasts, the enamel organ on the formative side of these cells being unchanged. This contrasts with the findings in other deficiencies, when other parts of the enamel organ are affected.

5. It is concluded that the enamel organ of continuously erupting teeth varies from part to part in its sensitivities to toxic substances and requirements of essential nutrients, these variations corresponding with the differing physiologic functions of the parts concerned.

The writer is indebted to Dr. O. E. Budtz-Olsen for carrying out the dialuric acid hemolysis tests.

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EFFECT OF SODIUM FLUORIDE ON ORAL LACTOBACILLI ISOLATED FROM THE RAT

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THE inhibition exerted by sodium fluoride on the growth and acid production of Streptococci, Lactobacilli, and other oral microorganisms was observed by Bibby and van Kesteren.[1] They found a reduction of approximately 50 per cent in acid production by Streptococci and Lactobacilli in media containing 100 p.p.m. NaF. A greater concentration was required, however, to achieve a bactericidal effect. Fitzgerald and Jordan[2] found that sodium fluoride and sodium fluorosilicate inhibited the growth of various Lactobacilli, as determined by the broth dilution method. The influence of fluoride on the growth and viability of staphylococci and yeast was demonstrated by Roberts and Rahn[6] to depend on the pH of the medium. Fluoride was most efficient in acid media. This was attributed to the existence of fluoride as hydrofluoric acid since the dissociation constant of HF is 7.2×10^{-4}.

This report deals with in vitro changes in the rate and extent of growth, as well as the bactericidal effects of sodium fluoride on various strains of oral Lactobacilli isolated from rats.

MATERIALS AND METHODS

All of the strains of Lactobacilli were obtained from Jordan and Fitzgerald.[3] The collection consisted of 20 homofermentative Lactobacilli and 20 heterofermentative strains. The homofermentative strains were: pp1, pp3, pp6, pp7, pp8, pp9, 6, 12, 16a, 21, 25, 33, 37, 39, 47, 49, 52, 57, 59, and 84 AT of which strains 84 AT, 16a, and possibly pp7 were *Lactobacillus acidophilus* or variants thereof; the remaining strains are types which have not been previously described in the literature. The heterofermentative strains were variants of *Lactobacillus fermenti*. These were strains 1, 2, 3, 7, 8, 9, 14, 16, 17, 18, 19, 20, 22, 23, 26, 30, 32, 37, 35A, and 38.

Stock cultures were maintained as stabs in Rogosa's SL Medium as modified by Fitzgerald and Jordan.[2] The medium was adjusted to pH 6.5 and dispensed into tubes containing approximately 0.1 Gm. $CaCO_3$. The tubes were then autoclaved for 10 minutes at 15 pounds. After incubation for 2 days at 37° C., the stock cultures were held at 5° C. and transferred about once a month.

Effects of Sodium Fluoride on Rates and Amount of Growth.—The test medium used for the study of the rates and amount of growth was the tomato juice broth (T.J.) described by Rogosa and Mitchell.[7] The double strength

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medium was adjusted to pH 6.8 unless otherwise stated. Solutions of sodium fluoride of varying concentrations, adjusted to the pH of the particular lot of medium being prepared, were added, and the medium was made up to final strength with distilled water, then autoclaved for 5 minutes at 15 pounds. The pH values recorded were taken after sterilization. No effort was made to maintain the pH after inoculation.

The experimental inocula were prepared by inoculating a T.J. broth tube from the stab and transferring twice in the exponential phase of growth. One drop of an 18-hour culture containing approximately 5×10^7 cells were used to inoculate each tube.

The extent of growth at varying fluoride concentrations after varying periods of incubation was measured as optical density at 660 mμ with the Beckman Model B Spectrophotometer, using matched 18 × 150 mm. Pyrex test tubes. The growth response of the organisms to varying fluoride concentrations was plotted as the logarithm of relative optical density (Figs. 1 and 3), which is defined as the logarithm of the ratio of the optical density obtained at any sodium fluoride concentration to that obtained with zero fluoride, times 100 ($\log OD/OD_0 \times 100$), after 48 hours' incubation.

Experiments on Bactericidal Activity of Sodium Fluoride.—In order to determine the bactericidal effects of sodium fluoride on the viability of cells 2 methods were used. In the first, the test was conducted at a given initial pH and the plate count method was used to enumerate surviving cells. In those experiments in which tests were conducted at several pH levels, the broth inoculation procedure was employed. In either case the cell suspensions were prepared by taking 10 ml. of a 20-hour-old culture, centrifuging, washing once in sterile distilled water, and standardizing by dilution with sterile water to an optical density of .04. For the plate count method this cell suspension was further diluted 1/10 and 1.0 ml. was added to a sterile tube containing 1 ml. veronal-acetate buffer, pH 6.9 (Michalis[4]), sodium fluoride at appropriate concentrations (0 to 1,000 p.p.m. NaF) and water to make a final volume of 10 ml. Each mixture was incubated at 37° C. A 1.0 ml. portion from each tube was removed at successive 20-minute intervals over a 2-hour period; each sample was then plated at appropriate dilutions on 10 replicate SL agar plates. These plates were incubated at 37° C. for 72 hours and the colonies counted. The data reported here are derived from the mean counts of 10 replicate plates.

The procedure followed for the second method, in which the effects of sodium fluoride at several pH levels were tested, was as follows: 1.0 ml. of the once-washed cell suspension was added to a sterile tube containing 1.0 ml. of citrate-phosphate buffer (McIlvaine[5]) of appropriate pH, NaF solutions of appropriate concentration and pH, and water to make a final volume of 10 ml. The mixture was incubated at room temperature. One-tenth ml. samples were then removed from each tube every half hour for 3 hours and inoculated directly into a tube containing 10 ml. T.J. broth. The latter tubes were then incubated at 37° C. for 48 hours, and any evidence of growth recorded.

RESULTS

Twenty strains each of homofermentative and heterofermentative oral Lactobacilli isolated from rats were tested for growth in the presence of varying sodium fluoride concentrations. Typical fluoride inhibition curves are shown in Fig. 1. Eight homofermentative strains (pp1, pp7, pp9, 16a, 25, 49, and 37) behaved like 84 AT and 12 (pp6, pp8, 6, 12, 21, 33, 29, 47, 52, 57, and 59) like pp3. All of the heterofermentative strains behaved similarly to culture 20. With all of the strains studied, the amount of growth was inversely proportional to fluoride concentration.

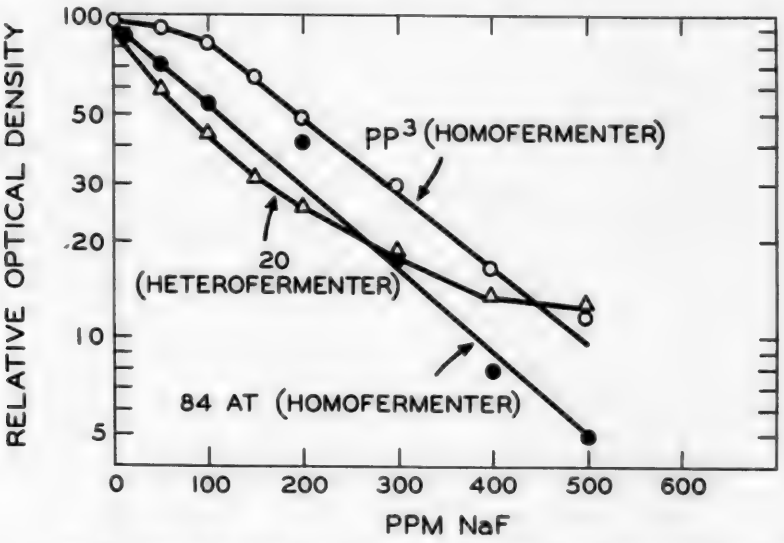

Fig. 1.—Effect of NaF on the growth of 3 types of oral Lactobacilli from the rat.

The effect on the rate of growth depended upon the strain employed. The growth rate of strain 84 AT, a homofermentative Lactobacillus (Fig. 2), was reduced by 50, 100, 200, and 300 p.p.m. NaF. At sodium fluoride concentrations of 400 or 500 p.p.m. there was an apparent lag, after which the rate of growth was commensurate to that of 300 p.p.m. In contrast, the growth rates of strain pp3, a second homofermentative organism, and strain 20, a heterofermentative organism, were decreased only slightly by as much as 500 p.p.m. sodium fluoride. In some strains, at concentrations of 300 to 500 p.p.m. sodium fluoride, lysis of cells apparently occurred after 9 to 24 hours, depending on the strain, as there was a decrease in the optical density.

Strains 84 AT, pp3, and 20 were further studied to determine the effect of the initial pH of the medium on their sensitivity to fluoride. It was found that for strain 84 AT, fluoride is more effective at lower pH levels, as shown in Fig. 3. The K values for the slopes of the curves of 84 AT calculated by the

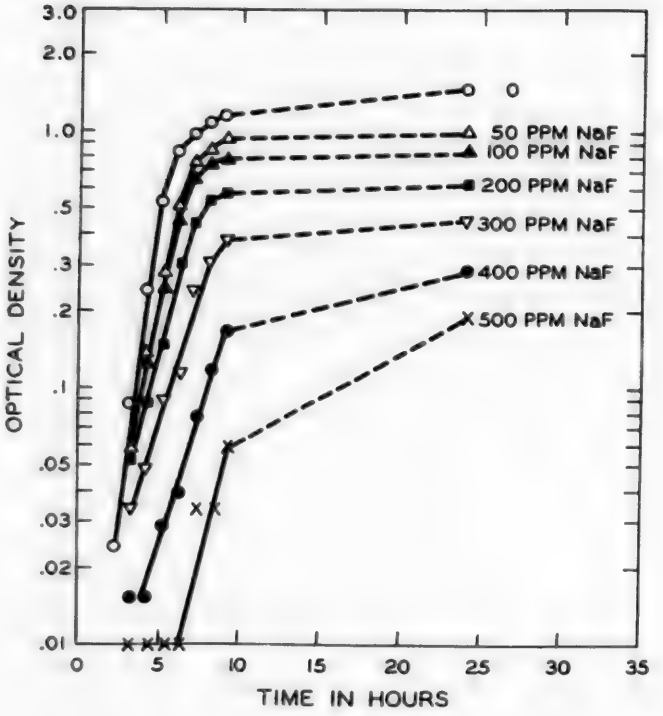

Fig. 2.

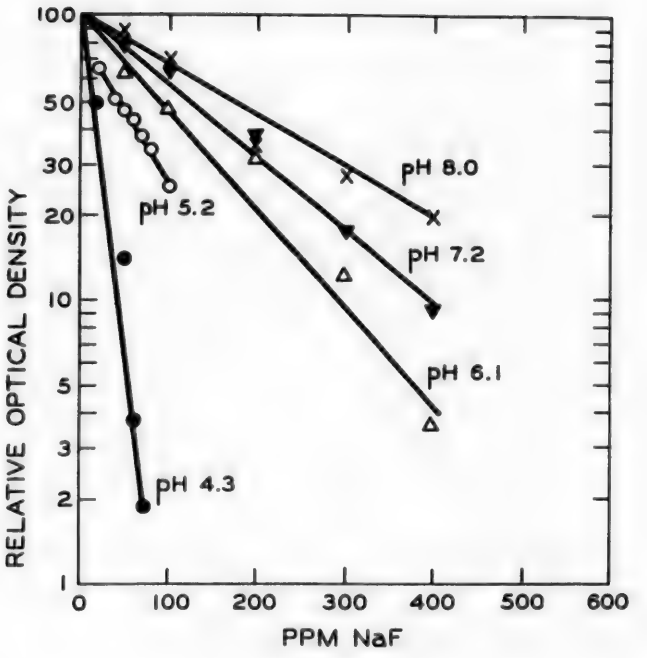

Fig. 3.

Fig. 2.—Effect of sodium fluoride on the growth rate of strain 84 AT.
Fig. 3.—The effect of pH on the inhibition of growth of strain 84 AT by sodium fluoride.

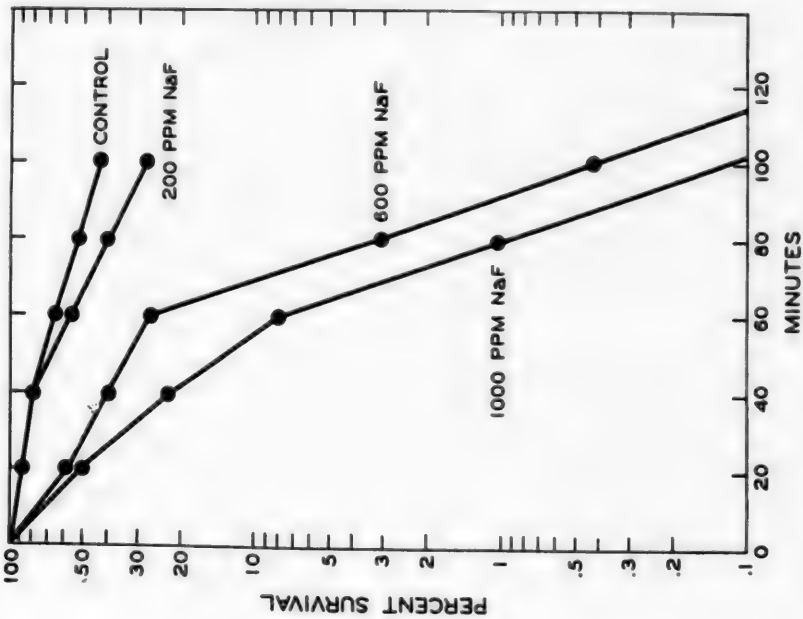

Fig. 5.

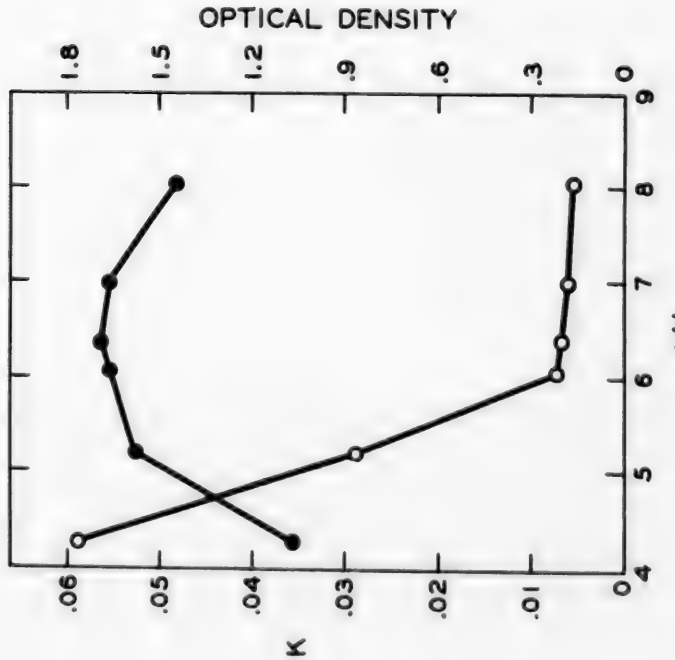

Fig. 4.

Fig. 4.—The effect of pH on the growth of strain 84 AT and the effectiveness of the growth inhibition by sodium fluoride. ○——○ K, ●——● = optical density of controls.
Fig. 5.—The bactericidal effect of sodium fluoride on cell suspensions of strain at 84 AT.

least squares method according to Snedecor[9] indicate the effectiveness of the growth inhibition by sodium fluoride under a given condition. The plot of the K values against the pH values is shown in Fig. 4. It can be seen that good growth occurred over the pH ranges indicated. On the assumption that this inhibition is due to the fluoride ions, the K values were used to calculate the concentration of fluoride (F) necessary to give 50 per cent inhibition for the various pH values which are shown in Table I. Results obtained from cultures pp3 and 20 are also tabulated. The shapes of the inhibition curves for strain pp3 and 20 over the pH range of about 8.0 to 5.0 were similar to those shown in Fig. 1. However at a pH of 4.3 to 4.4, the curves of both pp3 and 20 were similar to 84 AT.

TABLE I
CONCENTRATION OF SODIUM FLUORIDE NECESSARY TO GIVE A 50 PER CENT INHIBITION

84 AT			pp3		20	
pH	−K	p.p.m. F.*	pH	p.p.m. F.**	pH	p.p.m. F**
4.3	.0575	5.4	4.4	33	4.3	14
5.2	.0289	11.2	5.0	81	5.6	27
6.1	.0078	39.8	5.7	81	5.9	36
6.4	.0070	44.3	6.0	81	6.7	41
7.2	.0066	46.8	6.3	81	7.3	45
8.0	.0053	58.5	7.0	95	7.8	68

*F_{50} % = (.45) $-\frac{2.3}{K}$ log ½
= p.p.m. fluoride (F) to give 50 per cent inhibition of growth.
**Estimated from graphs.

The levels of sodium fluoride necessary to achieve a bactericidal effect on strain 84 AT are shown in Fig. 5. Nearly 2 hours were required to achieve a 99.9 per cent kill with bacterial suspensions of this strain exposed to 600 p.p.m. NaF at pH 6.9. Experiments were then performed with this strain to test the influence of pH values on the bactericidal effect of sodium fluoride. Results are shown in Table II. It can be seen that in the range of pH 4.0 to 6.0, the exposure of cells to 200 p.p.m. NaF for 2 hours was sufficient to prevent the growth of organisms in subcultures from aliquots tested; whereas at pH 7.0 or above, growth occurred from subcultures exposed to 1,000 p.p.m. NaF for 3 hours. In some experiments at pH 4.0, after 3 hours with no sodium fluoride added, subcultures failed to grow.

DISCUSSION

The various effects of fluoride on the growth of homofermentative and heterofermentative Lactobacilli are indicative of the distinct biochemical composition of these 2 groups. Moreover, within the homofermentative Lactobacillus group, there might be further distinctions reflected by their sensitivity toward fluoride, as seen in Fig. 1. At the lower fluoride levels, the type represented by pp3 is more resistant toward fluoride than that of 84 AT. However, at higher levels, the rate of inhibition parallels that of strain 84 AT.

The similar rate of growth of strain 84 AT at 500 p.p.m. sodium fluoride as compared to 300 p.p.m. is difficult to explain in view of the decrease in the total growth. The apparent lag period, which occurred at the higher sodium

fluoride concentrations, may not be a true lag since the initial number of cells introduced as inoculum may have been reduced by the bactericidal action of sodium fluoride. This possible reduction was not tested in the growth experiments.

The results of both the growth experiments and the bactericidal experiments are in agreement in indicating that the antibacterial activity of sodium fluoride for the oral Lactobacilli isolated from the rat is dependent on pH. The inhibitory effects of sodium fluoride are most pronounced at the lower pH levels presumably due to the higher concentrations of the undissociated HF in this range.

TABLE II
THE INFLUENCE OF pH ON THE BACTERICIDAL EFFECT OF SODIUM FLUORIDE ON CELL SUSPENSIONS OF STRAIN 84 AT

pH	NaF p.p.m.	EXPOSURE TIME 0	½	1	1½	2	2½	3
	0	+	+	+	+	+	+	–
	20	+	+	+	+	+	+	–
4	200	+	+	+	–	–	–	–
	600	+	+	+	+	–	–	–
	1000	+	+	+	+	–	–	–
	0	+	+	+	+	+	+	+
	20	+	+	+	+	–	+	+
6	200	+	+	+	–	–	–	–
	600	+	+	+	–	–	–	–
	1000	+	+	+	+	–	–	–
	0	+	+	+	+	+	+	+
	20	+	+	+	+	–	–	–
6.5	200	+	+	+	–	+	–	–
	600	+	+	+	+	–	–	–
	1000	+	+	+	+	+	–	–
	0	+	+	+	+	+	+	+
	20	+	+	+	+	+	+	+
7	200	+	+	+	+	+	+	+
	600	+	+	+	+	+	+	+
	1000	+	+	+	+	+	+	+
	0	+	+	+	+	+	+	+
	20	+	+	+	+	+	+	+
8.2	200	+	+	+	+	+	+	+
	600	+	+	+	+	+	+	+
	1000	+	+	+	+	+	+	+

SUMMARY

When varying concentrations of sodium fluoride were used to inhibit the growth of 20 strains each of homofermentative and heterofermentative oral Lactobacilli isolated from rats, 3 groups were distinguishable, as shown by the characteristic shapes of their growth inhibition curves. The homofermentative Lactobacilli were divided into 2 groups: in one, typified by strain 84 AT, a monomolecular reaction was observed and in a second, illustrated by strain pp3, a resistance to sodium fluoride through 50 p.p.m. was noted. The third group, represented by strain 20, was composed of heterofermentative Lactobacilli. The growth rate of strain 84 AT was reduced by 50, 100, 200, and 300

p.p.m. NaF. At concentrations of 400 or 500 p.p.m., there was an apparent lag after which the rate of growth was approximately the same as that at 300 p.p.m. With strains pp3 and 20, rates of growth were decreased only slightly through 500 p.p.m. NaF. The effect of pH on the inhibition of growth by sodium fluoride indicated that as the pH of the growth medium was lowered from pH 8 through 4, the inhibitory action of sodium fluoride against strains 84 AT, pp3, and 20 was increased. In bactericidal experiments 99.9 per cent of cells of strain 84 AT were killed when they were exposed to 600 p.p.m. NaF for 2 hours at pH 6.9. The bactericidal effect of sodium fluoride was increased as the pH of the testing mixture was decreased from pH 8 to 4.

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ETHYLENEDIAMINE VS. KOH-GLYCOL IN THE REMOVAL OF THE ORGANIC MATTER OF DENTIN

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EXISTING methods for isolation of the inorganic fraction of teeth involve either thermal ashing at temperatures in excess of 500° C. or wet ashing[1] at temperatures in excess of 200° C. Recently, however, Williams and Irvine[2] have described a method for the preparation of inorganic matrix of bone by the use of ethylenediamine as solvent for the organic components. These authors extracted the bone with a constant boiling aqueous solution of the diamine in a Soxhlet apparatus. A major feature of this extraction is the relatively low temperature at which it is carried out (118° C.). Bone prepared in this manner was examined by x-ray diffraction pattern and it gave, qualitatively, the same spacing as bone prepared by other methods. Williams and Irvine, on examination of the ground-extracted bone by electron microscope, could find no trace of collagen, which confirmed a Kjeldahl nitrogen analysis of less than 0.1 per cent.

The present investigation was undertaken in order to compare the extraction properties of alkaline ethylene glycol with those of ethylenediamine on human dentin.

EXPERIMENTAL

Sample Preparation.—One master dentin sample weighing approximately 10 grams was chosen for this study. The dentin was obtained from third molars of young adult males (17 to 21 years of age) and was crushed to pass a 60 mesh sieve screen and desiccated to constant weight over calcuim chloride at room temperature.

Ethylenediamine Extraction.—Five different portions of the dentin, each weighing approximately 0.5 Gm., were extracted with ethylenediamine according to the procedure described by Williams and Irvine.[2] During extraction each portion was contained in a Fisher Pyrex coarse-fritted extraction thimble. Twenty to thirty cycles, requiring from 4 to 5 hours, were allowed for each extraction. The Soxhlet flask contained 100 ml. of aqueous 80 per cent ethylenediamine solution and this was not changed from one extraction to the next. The temperature within the Pyrex thimbles during extraction was 93° C. The residues were washed by 3 to 5 cycles of water contained in a second Soxhlet apparatus. After dessication to constant weight over calcium chloride the residues were analyzed for calcium, phosphorus, and nitrogen content.

KOH-Glycol Extraction.—Another 5 portions of the master dentin sample were extracted separately with 30 ml. of a 3 per cent solution of potassium

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hydroxide in ethylene glycol, using the procedure as described by Crowell, Hodge, and Line.[1] Each extraction required a period of 1½ hours during which time the glycol solution was allowed to boil gently (200 to 205° C.). Residues were washed in boiling water for 30 minutes and the washing process repeated until the residues were free of potassium hydroxide (phenolphthalein). All decantations were made through the same Fisher extraction thimbles used for the ethylenediamine extractions, and the thimbles were suspended in the boiling water during the washing process. After washing, residues were transferred as quantitatively as our technic would allow into the thimbles. Residues were then desiccated to constant weight over calcium chloride, and phosphorus, calcium, and nitrogen determinations made.

Analytic Methods.—Calcium determinations were made according to the procedure described by Sobel and Sklersky[3] with modifications suggested by Sobel, Rockenmacher, and Kramer.[4]

Phosphorus determinations were carried out using the method of Fiske and Subbarow.[5] Determination of nitrogen was made by the microKjeldahl method as described by Ma and Zuazaga.[6]

RESULTS AND DISCUSSION

Calcium, Phosphorus, and Nitrogen Contents of the Various Residues (Table I).—Results of calcium, phosphorus, and nitrogen determinations of the residues, as well as percentage recoveries, are given in Table I. Mean values and standard deviations are shown for the residues of the 5 diamine and 5 glycol extractions.

These results indicate high, consistent recoveries of residues from ethylenediamine extractions. The loss of 22 per cent of matter during the extraction correlates with the findings of Losee, Leopold, and Hess[7] in their work on dentinal protein. It may be noted that the residues from the diamine extractions are higher than those obtained by thermal ashing. Hess* has noted that the difference could possibly be due to carbonate, since the carbonate content of dentin is of approximately this magnitude.

The percentage of dentin which could be recovered as residue from glycol extractions was lower than those from diamine extractions and highly unpredictable, as is shown by the range of variation.

The calcium contents of the various residues are in close agreement.

Results indicate a higher phosphorus content of residues from glycol extractions.

TABLE I. CALCIUM, PHOSPHORUS, AND NITROGEN CONTENTS OF THE VARIOUS RESIDUES

	WHOLE DENTIN	ASH OF DENTIN*	RESIDUE AFTER ETHYLENEDIAMINE EXTRACTION†	RESIDUE AFTER GLYCOL EXTRACTION†
Recovery (%)	--	73.8	78.0 ± 0.78	56.4 ± 7.95
Calcium (%)	27.0	34.6	33.5 ± 0.36	33.5 ± 0.66
Phosphorus (%)	13.0	17.3	16.8 ± 0.50	17.9 ± 0.58
Nitrogen (%)	3.3	0.07	0.17 ± 0.06	0.02 ± 0.02

*550° C. per 3 hrs.
†Means of 5 determinations.

*Personal communication.

Nitrogen contents for the residues of diamine extractions are somewhat greater than those of residues from glycol treatment, but are still within the 0.1 per cent range reported by Williams and Irvine in their work with bone. Furthermore, it is not known at this time whether this nitrogen is representative of organic matter retained or whether it is the result of occluded or adsorbed ethylenediamine.

Percentages of Initial Calcium, Phosphorus, and Nitrogen Content Recovered in the Residues (Table II).—Values given in Table II represent the initial calcium, phosphorus, and nitrogen contents of the dentin which were retained in the residues after the extraction procedures had been carried out.

Residues of the diamine extractions compare favorably with residues from the thermal ashing of dentin in every respect studied except for nitrogen. As stated above, this retention of nitrogen may, or may not be due to the adsorption or occlusion of ethylenediamine by the residues.

TABLE II. PERCENTAGES OF INITIAL CALCIUM, PHOSPHORUS, AND NITROGEN CONTENT RECOVERED IN THE RESIDUES*

	ASH OF DENTIN	RESIDUE AFTER ETHYLENEDIAMINE EXTRACTION	RESIDUE AFTER GLYCOL EXTRACTION
Calcium	95	97	70
Phosphorus	98	100	78
Nitrogen	1.6	4	0.3

*Based on mean values shown in Table I.

The low recoveries from the glycol extractions are reflected here in the low values of total calcium and phosphorus retained in the residues.

It may be noted that residues from glycol extractions also appear to have retained some 10 per cent more of their initial phosphorus content than of their initial calcium. This difference persisted even after a sulfuric acid-peroxide oxidation was done prior to the phosphate determinations.

SUMMARY AND CONCLUSIONS

Certain extraction properties of alkaline ethylene glycol have been compared with those of ethylenediamine on human dentin.

It appears that use of ethylenediamine for the extraction of dentinal organic matter will permit the recovery of a greater percentage of the mineral fraction than can be accomplished by the use of alkaline ethylene glycol.

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PUNCHED-CARD RECORDING OF CARIOUS LESIONS OF BOTH DENTITIONS OF MAN

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INTRODUCTION

FOR definitive study of the causes of dental caries, the data of carious attack should be available in particulate form for all areas of all teeth of both dentitions of man. Furthermore, the data of all suspected causal or preventive factors should also be kept separate so far as that is possible.[1]

PRINCIPLES

This report is concerned with a method of reducing individual dental records to a form, namely, a single punched card of the Hollerith type that permits (1) the ready analysis of a population for simple frequency of carious attack on any surface of any of the primary and permanent teeth of man, (2) study of association of decay of any groups of 2 or more dental areas, and (3) study of frequency of and associations of other oral conditions.

In order to record all of the data of caries in 52 teeth as well as to give description of the subjects and other oral conditions on a single card, the following principles have been used:

1. If the object of a study is prevention of dental caries then interest is in the frequency of noncarious areas. This can be shown best by recording carious areas. Subsequent fate of an area, whether decay spreads to other surfaces, whether the lesion is restored or the tooth is lost, is not pertinent to prevention. If two or more examinations are made, new carious lesions, without estimate of size of cavities, can be analyzed by these methods. If the study is concerned with progress of caries then sizes of cavities must be estimated and other methods than these presented must be used.

2. Position in the dental arch has been used rather than reference to a specific tooth. Thus, a space is occupied by either a primary or a permanent tooth or it is vacant.

3. The pattern of carious attack on a tooth is given by a single number which represents the combination of all possible carious lesions. This number is derived from the formula $2^n - 1$ in which "n" is the number of surfaces which commonly are attacked by caries in a specific tooth. For example, if a tooth may become carious in 4 areas there are $2^4 - 1$, or 15 combinations of decay. (See Table I for combinations of decay in 7 areas.)

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TABLE I

GENERAL ARBITRARY CODES FOR SEVEN AREAS OF CARIOUS ATTACK, COLUMNS 14 TO 65, INCLUSIVE

AREAS	CODE NO.	AREAS	CODE NO.	AREAS	CODE NO.	AREAS	CODE NO.
m	1	mb12	36	d1	68	b2	100
md	2	mb13	37	d2	69	b3	101
mb	3	mb23	38	d3	70		
mp	4	mp12	39			bp1	102
m1	5	mp13	40	dbp	71	bp2	103
m2	6	mp23	41	db1	72	bp3	104
m3	7	m123	42	db2	73	b12	105
				db3	74	b13	106
mdb	8	mdbp1	43	dp1	75	b23	107
mdp	9	mdbp2	44	dp2	76		
md1	10	mdbp3	45	dp3	77	bp12	108
md2	11	mdb12	46	d12	78	bp13	109
md3	12	mdb13	47	d13	79	bp23	110
mbp	13	mdb23	48	d23	80	b123	111
mb1	14	mdp12	49				
mb2	15	mdp13	50	dbp1	81	bp123	112
mb3	16	mdp23	51	dbp2	82		
mp1	17	md123	52	dbp3	83	p	113
mp2	18	mbp12	53	db12	84		
mp3	19	mbp13	54	db13	85	p1	114
m12	20	mbp23	55	db23	86	p2	115
m13	21	mb123	56	dp12	87	p3	116
m23	22	mp123	57	dp13	88		
				dp23	89	p12	117
mdbp	23	mdbp12	58	d123	90	p13	118
mdb1	24	mdbp13	59	dbp12	91	p23	119
mdb2	25	mdbp23	60	dbp13	92		
mdb3	26	mdb123	61	dbp23	93	p123	120
mdp1	27	mdp123	62	db123	94		
mdp2	28	mbp123	63	db123	95	1	121
mdp3	29					12	122
md12	30	mdbp123	64	dbp123	96	13	123
md13	31					123	124
md23	32	d	65	b	97	2	125
mbp1	33					23	126
mbp2	34	db	66	bp	98	3	127
mbp3	35	dp	67	b1	99		

4. Carious attack can be arbitrarily assigned to a set of susceptible areas, as did Black,[2] but the code number can be reduced empirically to description of less than 100 combinations for any tooth.

PROCEDURES

For the practical transfer of individual dental findings to punched cards, records of about 4,300 American Samoan children and young adults* have been used.[3] The data were coded directly on the original record forms and the cards were punched by an operator using a template bearing guides to the columns to be punched in the cards and masking all other aspects of the records. One of the principal objectives of this study was to design an original record form to facilitate coding for the more ready transfer of data to the punched card.

For this study on Samoan children, the data descriptive of the examinees and of the examiners required 13 columns, as shown in Table II. In general,

*The authors are indebted to Commander Fred L. Losee of the Naval Medical Research Institute for the use of the original records.

TABLE II

DESCRIPTION OF SUBJECTS AND EXAMINING PERSONNEL

SUBJECT MATTER	COLUMNS
Examinee, sex	1-4
Examiners and recorders	5
Name of school	6, 7
Name of village	8, 9
Age in months	10-12
Type of dentition, caries or no caries	13

fewer columns would be required but in this study subjects were drawn from over 90 villages and there were 35 schools with names differing from the names of the villages.

In Columns 14 to 65, inclusive, the records of dental caries were punched as follows: primary tooth, upper row, commonly designated as "12"; missing tooth, second row, designated as "x"; no punch in either of these rows indicated that a permanent tooth was present in the space. If the tooth was carious, a number was punched in one or two columns according to an empirically determined number. The general system of determining the number from the formula $2^n - 1$ is shown in Table I.

It was recognized that all teeth were susceptible to decay in both mesial and distal surfaces. The letter "m" was assigned in the code for carious attack in mesial surfaces and "d" for distal surfaces. The letter "b" was used for buccal and labial surface decay. Since the typewritten letter "l" is the same as the number "1," the letter "p" (palatal) was chosen to represent all lingual decay. The numbers "1," "2," and "3" were used for decay in mesial, central, and distal pits of occlusal surfaces, respectively.

It is indicated by Table I that there are 127 combinations of decay in a molar tooth presenting 3 occlusal pits and 4 lateral surfaces. Empirically, many of these combinations were not found and the number was reduced in all molar teeth to less than 100, thus obviating the use of more than 2 columns for each tooth.

If the tooth was free of carious attack, no number was punched.

In all practical studies unusual conditions are found. This was provided for by punching "9" in single columns and "90" in double columns so reference could be made to original records for interpretation. If only the roots of a tooth remained, "8" was punched in single columns and "80" in double columns.

For coding, inspection was first made of the mesial surface, followed by distal, buccal, and lingual and the mesial, central, and distal pits. The code was very quickly and easily found. By inspection of the records for the molars, the arbitrary codes were readily reduced to empirical codes if it was necessary to obtain numbers less than 100.

In the foregoing, all numbers punched other than in "12" and "x" were unique and, hence, exclusive of other numbers. In the remainder of the columns multiple punching was used.

In Table III is shown the use made of Columns 66 to 80, inclusive.

TABLE III

RECORDS OF ORAL CONDITIONS OTHER THAN DENTAL CARIES

COLUMN	CONDITION AND DESCRIPTION*
66	Calculus: type, degree and location
67	Stains: degree, color, location
68	Wear: degree, location, special features
69	Cracked plates: teeth affected
70	Buccal pits: teeth affected
71	Deep pits and fissures: locations
72	Orthodontic descriptions including supernumerary teeth
73	Lingual version: teeth affected
74	Labial version: teeth affected
75	Opaqueness: types and special cases
76	General cleanliness
77	Gingivitis
78	Paradentosis
79	Occlusion (mandibular)
80	Mottling

*We are indebted to Dr. L. E. Kuentzel of Wyandotte Chemical Corporation for advice on multiple punching of the cards in these columns.

DISCUSSION

It is to be emphasized that the recording of the decisions of the dental examiner was the sole purpose of this study and the decisions were entirely accepted as bona fide.

Single restorations extending into 2 or more empirical areas have been treated as primary decay in each area. Such restorations cannot otherwise be treated unless the records indicate extension for prevention rather than for repair. Also, prophylactic fillings must necessarily be treated as decayed areas unless records indicate otherwise.

It is also to be emphasized that herein are presented principles rather than a finished method for recording caries findings from diverse studies.

SUMMARY

A method has been described for recording in a single punched card oral conditions involving all the teeth of both dentitions of man, including mixed dentition. The complete condition of each tooth is shown by a single number representing an empirically determined combination of carious attack.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the British Division
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British Division

I. Index of Participants and Sequence Numericals for Corresponding Abstracts

Bisset, K. A., 25; Blake, G. C., 18; Brain, E. B., 10; Cooke, B. E. D., 1; Crabb, H. S. M., 15; Darling, A. I., 15; Davis, G. H. G., 25; Dixon, A. D., 13; English, J. A., 30; Farmer, E. D., 2; Fearnhead, R. W., 19; Gairns, F. W., 9; Greulich, R. C., 22; Hartles, R. L., 28, 26; Hayward, A. C., 25; Hitchin, A. D., 5; Hodson, J. J., 3; Holloway, P. J., 16; Hughes, Shirley G., 17; Jackson, D., 4; Jenkins, G. N., 24; Kerr, A. C., 23; Kleinberg, I., 24; Kramer, I. R. H., 7, 8; Leach, S. A., 20; Mackenzie, E. B., 7; McLean, J. W., 7; Mellanby, May, 16; Noble, H. W., 6; Rowles, S. L., 10; Russell, L. H., 8; Scott, J. H., 11; Stack, M. V., 29; Staple, P. H., 27; Ten Cate, A. R., 14; Tonge, C. H., 12; Wagg, B. J., 26; Weidmann, S. M., 21.

The British Division held its fourth meeting at the School of Dental Surgery, University of Liverpool, on Friday, April 13, and Saturday morning, April 14, 1956. Thirty papers and 7 demonstrations were presented and the meeting was attended by 85 members and visitors, this attendance being 30 per cent larger than at last year's meeting.

1. Oral Epithelial Nevi. *B. E. D. Cooke, Department of Dental Medicine, Guy's Hospital, University of London.* There are nevoid disturbances either present at birth or developing later in life in which hyperplasia of the prickle cells occurs without the presence of ordinary nevus cells. It gradually progresses to a certain point and then remains stationary. Being symptomless, it may be discovered on any part of the oral mucosa, at any age, and in either sex. The white patch has a wrinkled appearance with tesselation or wartiness, and is often symmetrical. Two main histologic appearances are observed; either there is an increased rate in the production of keratin, giving the keratin layer a definite basket-weave pattern, or there is a failure of the keratin to separate. The underlying corium is normal. A peculiar dyskeratosis below the granular layer may be seen that would appear to impair the mechanical properties of the epithelial layer, so that it splits, exposing the underlying corium.

2. Recurrent Ulcerations of the Oral Mucosa. *E. D. Farmer, Department of Parodontal Diseases, University of Liverpool.* The patients examined

in this preliminary survey have been divided into 2 main classes; those with and those without a previous history of acute herpetic stomatitis. Fifteen cases with a confirmed diagnosis of acute herpetic stomatitis reported having had ulcers or vesicles in the mouth over periods ranging between 6 months and 2 years after the primary infection. Ten of these had lesions only in the mouth and 5 had lesions in the mouth and on the lips. None of these patients had experienced ulcers in the mouth prior to the acute infection. A virus was not isolated from the 2 recurrent lesions where this was attempted. A biopsy taken from one case where the lesion appeared 5 weeks after the primary infection showed vesicle formation and typical herpetic intranuclear inclusion bodies in the involved epithelial cells. Eighteen patients with recurrent oral ulcerations but with no previous history of an acute stomatitis were examined. Attempts at virus isolation, using the chorioallantoic membrane of the 12-day-old embryonated egg, were unsuccessful. Histologic examination of early lesions in 18 cases showed that there were at least 3 different types of lesions: (1) a nonspecific type of ulcer; (2) a proliferation of the epithelial cells, thickening of the cell membrane, pyknosis or karyolysis, and a granular appearance of the cell contents; and (3) the formation of an intraepithelial vesicle which may be accompanied by the changes recorded under 2. The relationship between these 3 types of lesion is at present uncertain.

3. ADAMANTINOMAS OF THE MANDIBLE SHOWING MUCOEPIDERMOID VARIATIONS. *J. J. Hodson, Department of Oral Pathology, Dental School, University of Sheffield.* Only one adamantinoma showing mucous changes appears to be mentioned in the literature (McGregor, *Acta Radiol.* **16**: 254, 1935). In 3 cases of classical adamantinomas, mucous changes have been found, the character of which, however, does not warrant them being classified as a separate group, but rather as a further structural variation emphasizing the pleomorphic character of these tumors. The appearances are compared with mucoepidermoid patches in otherwise typical mixed salivary tumors and the group known as mucoepidermoid carcinomas. The extent of mucous change in the adamantinomas varies considerably in different tumors, ranging from a few cells to extensive mucus-filled cysts. Glycogen, amongst other materials, may also be present and must be distinguished from mucin. Poorly staining, denatured mucin, eosinophilic splashes and globules of mucin, and also metachromatic mucin occur and are identical in appearance to those seen in mixed salivary tumors. The mucous cell metaplasia seems to take place chiefly after squamous differentiation, and often enlargement, of the stellate and intermediate "basal cells" of the tumor follicles. Less frequently it occurs direct from "stellate cells." It may also occur in the primary (i.e., nonmetaplastic) squamous epithelium seen in some adamantinomas. In addition there are mucous microcysts similar to those recently described in certain mucoepidermoid odontogenic cysts of the jaws. (Hodson: *Proc. Roy Soc. Med.* **49**: 637, 1957.) In some of the larger cysts the contents may consist entirely of mucous material or globules. No conclusions can be drawn yet regarding the clinical behavior of adamantinomas showing these features.

4. Initial Considerations on the Phenomenon of Arrested Dental Caries. *D. Jackson, Biological Research Unit, School of Dentistry, University of Leeds.* The investigation was initiated to determine the nature of that condition in which an extensive caries lesion eventually ceases leaving a hard, sound, and invariably stained dentin surface which appears to have good resistance to further attack. A gross section of these teeth shows a well-circumscribed area of stained dentin which is optically different from normal dentin. In thin ground sections the condition is analogous to the phenomenon which has been termed "metamorphosed dentin." A transverse section of the tubules in this area shows that they are partially or completely occluded with mineral salts. In decalcified sections the dentin appears to be normal. X-ray diffraction pictures of this changed dentin show a better crystalline structure than is present in adjacent normal dentin.

5. Occlusion in Sheep—Some Breeding Experiments. *A. D. Hitchin, Dundee Dental School, Queen's College, University of St. Andrews.* The different types of occlusion of the incisors against the maxillary pad in sheep have already been described. In Type 1 the incisors occlude against the crest of the pad, producing a right angle wear from attrition; whereas in Type 2, common in the Cheviot breed, they occlude onto the front of the pad and become worn to a sharp chisel bevel (Hitchin, *D. Rec.* **58**: 251, 1948). Experiments have been made to try to control the type of occlusion in Cheviot flocks by breed selection. Random samples of Cheviot lambs from Nithsdale showed 47.7 per cent and amongst the ewes 9.9 per cent with Type 1 occlusion. Cheviot tups with Type 1 occlusion were found to serve these Cheviot ewes. Five separate experiments were carried out in this manner with 4 different tups. As a result, a total of 246 lambs was obtained, of which 177 displayed Type 1 occlusion and 69, Type 2. Thus, 71.5 per cent of the lambs had Type 1 occlusion compared with 47.7 per cent in the random samples. There are, therefore, hereditary factors controlling occlusion in sheep, which should not be ignored in breed selection.

6. The Occurrence and Identification of Cotton Fibers in Operative Wounds. *H. W. Noble, Dental Anatomy Department, University of Glasgow.* The tissue reaction of a healing operative wound to nonabsorbable mineral particles such as talcum powder has been extensively reported. Less attention has been paid to the possible inclusion of microscopical fibers of cotton wool during an operation, and to the tissue reaction which they provoke if not removed. Investigations have been made upon the factors which lead to the wound contamination by cotton fibers and the difficulties associated with their subsequent identification in the healing tissue. It is concluded that such contamination may occasionally occur and that it may cause delayed healing of the wound.

7. Histologic Studies on the Use of Plastic Emulsions for Cavity Lining. *I. R. H. Kramer and J. W. McLean, Institute of Dental Surgery, University of London, and Surg. Cdr. (D) E. B. Mackenzie, R.N.* Plastic emulsion paints

(p.e.p.) can be applied to skin repeatedly without causing irritation, and they adhere firmly to most surfaces, forming a slightly pliable film of uniform thickness. In addition they can be diluted with water and consequently are unaffected by slight moisture contamination during application. Therefore, their possible usefulness as cavity lining materials was investigated, especially in relation to the self-polymerizing acrylic resins. The experiments were designed to determine: (1) whether the material caused pulpal irritation, and (2) whether pulp reactions to the filling resins would be prevented. Cavities prepared in noncarious human teeth were lined with p.e.p. and filled with (a) amalgam, (b) a resin activated with paratoluene sulphinic acid, and (c) a resin activated by a benzoyl peroxide-lauryl mercaptan system and also containing methacrylic acid. Histologic examinations showed that in Group A the pulps showed no inflammatory reaction. However, in Group B the pulp reactions did not differ significantly from those encountered when the same resin was used unlined (McLean and Kramer, *Brit. D. J.* **93**: 255, 1952), while in Group C p.e.p. did not prevent the development of the characteristic pulp reactions to methacrylic acid (Kramer, *J. D. Res.* **34**: 782, 1955). It was concluded that, although free from significant irritant properties itself, this particular type of p.e.p. does not form an effective barrier against the irritant components of two self-polymerizing resins. Preliminary experiments with another type of p.e.p. suggest that modification of the formula may result in the development of a material which is a more effective barrier.

8. Observations on the Vascular Architecture of the Dental Pulp. *L. H. Russell and I. R. H. Kramer, Department of Dental Pathology, Institute of Dental Surgery, University of London.* Using a method previously described (Kramer, *Anat. Rec.* **3**: 91, 1951), pulpal blood vessels were injected in extracted carious and noncarious deciduous and permanent teeth. It was found advantageous to filter the perfusing fluid (Keller and Cohen, *Oral Surg.* **8**: 539, 1955). Satisfactory injections were regularly obtained with fully formed permanent teeth, and with deciduous teeth even when there was marked root resorption. Permanent teeth with open apices gave less consistent results, possibly due to a tendency of the apical vessels to collapse. The following features were observed in addition to those reported by previous workers. In many root canals the subodontoblastic plexus is present on one aspect only. In permanent teeth there are often numerous lateral canals connecting the root canal to the periodontal membrane; these usually contain a pair of vessels, one large and one small. Where roots are flat and have more than one canal, as in lower molars, or where they are fused, a complex of vessels is seen passing from one root canal to the other across the dentine bridge. Sometimes, a large vessel pierces the wall of the bridge and communicates with the vascular systems of both root canals. Mainly in the deciduous teeth, large vessels were noted passing through the walls of the root canals and having branches running in both coronal and apical directions. In the pulp chamber there appears to be a full anastomosis between the vessels of each root, rather than separate closed vascular systems.

9. The Sensory Nerve Endings of the Human Gingiva, Palate, and Tongue. *F. W. Gairns, Institute of Physiology, University of Glasgow.* Descriptions of the sensory nerve endings of the human gingiva and palate have already been published (Gairns and Aitchison, *D. Rec.* **70**: 180, 1950, and Gairns, *Quart. J. Exp. Phys.* **40**: 40, 1955); more recent work has been directed to the endings in the tongue. The endings in these 3 regions are similar. In all 3 sites the endings are of 2 main types: (1) free nerve endings in the epidermis and upper dermal zone, and (2) organized endings in the dermis lying mainly in the dermal papillae or immediately below them. The free nerve endings are extremely profuse and may arise from either myelinated or nonmyelinated nerve fibers. The organized endings are either encapsulated or nonencapsulated. The encapsulated endings are typical Meissner corpuscles or Krause end-bulbs such as are found in the glabrous skin. The unencapsulated types are usually whorls or skeins of varying size and shape. Several may be found in a single papilla. Many of the organized endings have a special feature: they exhibit fine fibrils (ultraterminal) which pass from the actual ending in the papilla into the epithelium and run there in a straight unbranched course to the outermost layers. It is suggested that these "ultraterminal" fibrils are probably present only in moist mucous membranes since there is no evidence for their occurrence on other surfaces of the body. Recent work (Nisbet, *J. Physiol.* **130**: 3P, 1955), indicates that similar unbranched fibrils passing into the epithelium from the dermis are present in the moist muzzles of cattle.

10. Silver Staining of Nerves in Urea Solutions. *S. L. Rowles and E. B. Brain, Department of Dental Pathology, University of Birmingham.* The silver staining of nerves by Ungewitter's method (*Stain Technol.* **26**: 73, 1951) utilizes impregnation by silver nitrate solution containing a large concentration of urea. Variation in staining obtained with this method prompted an investigation of the changes occurring in the impregnating solution. Nerve staining appears to depend on attaining suitable pH and silver ion concentration levels, and may be enhanced by the presence of silver cyanate. A modified impregnating solution consisting basically of a saturated solution of silver cyanate in buffered (pH 5.5 to 6.5) urea has given fairly consistent nerve staining with a variety of formalin-fixed, distearate-embedded human teeth. Impregnation with other solutions saturated with silver cyanate has also given useful results. Consideration is being given to the mechanism of the nerve staining process in these solutions.

11. The Nerve Supply of the Upper Teeth. *J. H. Scott, Anatomy Department, Queen's University, Belfast, N. Ireland.* There is lack of agreement in the textbooks regarding the presence of the middle superior dental nerve in man. The relationship of the superior dental nerves to the teeth was studied in fetal material, and the neurovascular canals were investigated in a series of adult maxillary bones. A middle dental nerve was found to be present in a large number of cases (see also Olsen, Teuscher, and Vehe, *J. D. Res.* **34**: 413, 1955). It may be present on one side of the face and absent on the other.

Unlike the anterior dental nerve, which is remarkably constant in its course (Wood-Jones, *J. Anat. Lond.* **73**: 583, 1939), the middle and posterior nerves show a wide range of variation in their position relative to the teeth and the maxillary antrum.

12. Observations on the Dental Lamina in Man. *C. H. Tonge, Department of Anatomy, Medical School, Newcastle-upon-Tyne.* Serial sections cut sagitally, horizontally, and coronally of human fetal material between 6 weeks and 5 months have been studied. As the dental lamina forms, that part of it which lies deepest within the connective tissue consists of a core of closely packed spherical cells lined by tall columnar cells. Nearer the surface the lamina comprises loosely arranged polyhedral cells lined by low columnar or cuboidal cells. In the vicinity of the deciduous mandibular first incisor, a corrugation of the surface cells with a concentric arrangement of the central cells was observed in the zone of junction between the labial and main parts of the dental lamina. This retrogressive change appears coincident with cellular differentiation of the enamel organ. During separation of the enamel organ from the labial part of the dental lamina, the frequent persistence of a deep connection to the outer enamel epithelium is observed in the stage preceding amelogenesis. Disintegration of the superficial parts of the dental lamina of the deciduous dentition in the fourth and fifth months is marked by islet or strand formation. These islets, comprising a core of polyhedral cells lined by cuboidal cells, may disappear, remain latent, or undergo proliferative degeneration characterized by an enlargement and concentric arrangement of their cellular center with a flattening of their lining cells. Cystic degeneration is commonly observed. The deeper parts of the lamina and the area of separation of the successional tooth buds break up by forming small cellular clusters, or networks, or individual cells.

13. Early Development of the Maxilla in Mammals. *A. D. Dixon, Department of Anatomy, University of Manchester.* Stained serial sections of the head region of 67 embryos and fetuses enabled the early stages of formation of the maxilla to be studied in representatives of the Orders Artiodactyla, Rodentia, Carnivora, and Primates. In the sheep, typical of mammals in which the premaxilla is separate from the maxilla throughout intrauterine life, wax reconstructions of the maxilla and related structures in embryos of 27, 30, and 35 mm. CR length show that bone deposition begins with the formation of a single center of ossification, which is closely related topographically to the angle formed by the infraorbital and anterior superior dental nerves. As extensions of this primary neural element, frontal, zygomatic, palatal, and alveolar processes are formed, establishing secondary relationships with the nasal capsule and the dental lamina. This is the basic pattern of development in other mammals, despite wide variations in facial contours, the primary bone-nerve relationship occurring so constantly as to suggest induction of the maxilla by nervous tissue. Secondary or accessory cartilage enters into the formation of the maxilla at certain stages in some mammals, particularly in

the zygomatic and alveolar regions of rodents and man. This cartilage is often of a temporary hypertrophic type associated with growth pressures, in contrast to the hyaline growth cartilage of the mandibular condyle. It is suggested, however, that maxillary and mandibular bone development is not dissimilar.

14. Observations on the Cells of the Enamel Organ. *A. R. Ten Cate, Anatomy Department, London Hospital Medical College, University of London.* Previous investigators have described cells of the stratum intermedium intermingling with the ameloblasts before the onset of calcification. Additions to the ameloblast layer by the cells of the stratum intermedium after the initiation of calcification have also been reported (Lehner and Plenk, Mollendorff's Handb. d. mikrosk. Anat. d. mensch., Vol. 3, Berlin, 1936). The present investigation concerns observations made on the relationship between the cells of the stratum intermedium and the ameloblasts, in human and other mammalian material. Cells having the characteristics of "kionoblasts" (Symons, *Brit. D. J.* **98**: 273, 1955) were observed among the ameloblasts. The nuclei of these cells were often situated in the stratum intermedium with their narrow elongated cell bodies passing through the entire width of the ameloblast layer. These cells are connected, by means of cytoplasmic processes, with the cells of the stratum intermedium. Intermediate stages in the histogenesis of this type of cell were identified when the internal enamel epithelium, from the morphogenetic stage up to the stage of enamel matrix production, was examined. It is suggested, therefore, that the cells called kionoblasts by other workers may have their origin in the stratum intermedium.

15. X-ray Investigations of Developing Dental Enamel. *H. S. M. Crabb and A. I. Darling, Dental School, University of Bristol.* The characteristic radiation of copper has been used to examine thin ground sections of developing dental enamel to show the pattern of calcification. By the use of aluminum penetrometer wedges a quantitative assessment of calcification can be made. In preliminary investigations of a number of deciduous incisor and molar teeth from full-term human fetuses, it has been found that a marked band of radiopacity can be shown in the enamel close to the amelodentinal junction. This band seems to be formed before the full adult thickness of the enamel has been attained. Calculations so far based on a visual estimation of comparative radiopacities of penetrometer wedges and tooth sections suggest that this band of radiopacity at the amelodentinal junction represents calcification of the order of 70 per cent by volume, while the outer enamel at this stage appears to show a diminishing degree of calcification from within outward. In an examination of ground sections of an unerupted second molar of a gibbon, differences in radiopacity related to the striae of Retzius have been found in the outer third of the enamel.

16. The Effect of Vitamin A on the Growth of Tooth Germs in Tissue Culture. *May Mellanby and P. J. Holloway, Nutrition Building, National Institute for Medical Research, London.* Pairs of lower first molar tooth germs from 17 to 18-day embryo rats were cultured on cock plasma clots using the

method described by Fell and Mellanby (*J. Physiol.* **116**: 320, 1952) for embryonic bones. One from each pair was explanted on control medium, and the other, the same medium to which sufficient vitamin A had been added to raise the concentration to about 1,350 i.u. per 100 ml. Germs cultured on hyper A clots grew much more slowly than the control germs, and although after four days' cultivation some sign of cusp formation was present, after fourteen days this was no longer apparent. The controls were normal in shape but cusp formation was retarded at all the stages examined. Histologic sections showed that the control germs cultured for 14 days had reached a stage that would have been attained after 5 to 6 days in vivo. From the early bell stage they became fully differentiated germs with some tubular dentin matrix. Germs grown on the hyper A medium for 14 days appeared healthy but showed relatively little differentiation, especially in the enamel organs. The stellate reticulum present on the fourth day of culture was often missing altogether by the fourteenth day. The ameloblasts were cubical or oval, and their nuclei had failed to migrate to the distal poles of the cells in contrast to those of the control germs. Vitamin A appears to exert a direct action upon the developing tooth germ, the tissues most affected being those of epithelial origin.

17. Experiments on the Calcification of Teeth. *Shirley Glasstone Hughes, Department of Anatomy, University of Cambridge.* Experiments on the calcifiability of dentin caps have been made with a view to studying the ground substance. The caps have been digested with testicular hyaluronidase, and then immersed in calcifying solutions. Calcifiability is unchanged after this preliminary treatment although the metachromasia of the dentin matrix is thereby reduced. These experiments suggest that the mucopolysaccharide material thus removed plays no important role in calcification. Further experiments have suggested, however, that some acidic substance in the dentin matrix plays a part in calcification, for the addition of protamine sulphate to a calcifying solution greatly reduces its effectiveness. In this experiment protamine, which is strongly basic, competes with the calcium for the acidic substance of the dentin matrix. Again preliminary digestion with hyaluronidase does not affect the course of this experiment.

18. A Method for the Demonstration of Diffusion Pathways in Enamel, Dentin, and Cementum in the Intact Tooth. *G. C. Blake, Department of Dental Pathology, Institute of Dental Surgery, University of London.* For the demonstration of diffusion pathways in cementum, dentin and enamel silver nitrate impregnation has proved difficult to control and unpredictable in its results, while stains generally give a weak coloration and stand acid decalcification poorly. The method described below has been used to trace movements of an inorganic ion (Hg) in the dental tissues and has proved an effective way of demonstrating tubules and lateral branches in dentin, lacunae, and canaliculi in cementum, and lamellae and tufts in enamel. The method has also assisted in the investigation of changes in enamel and dentin due to caries. Undecalcified teeth, either formalin-fixed or fresh, are immersed in 5 per cent mercuric chloride for 2 to 12 weeks, according to the degree of penetration required. Mercuric ions are then fixed in the tooth substance as black mercuric

sulphide by immersion in ammonium sulphide. After precipitation of the mercuric sulphide, ground sections may be prepared or the specimen may be decalcified and paraffin sections cut: these may be stained by any of the usual histologic technics. The advantages of the method are the ease with which it may be controlled, the density of the impregnation, and its resistance to acids used for decalcification. This makes it possible to see fine detail in impregnated areas in paraffin sections of normal thickness and to apply the usual histologic staining technics.

19. Preparation of Ultrathin Sections of Calcified Tissues by Means of a Diamond Knife. *R. W. Fearnhead, Department of Dental Histology, London Hospital Medical College.* Diamond knives were used to cut ultrathin (500 to 200 Å) sections of calcified dental tissues. Two diamond wedge knives, both with an edge angle of 70 degrees and a blade length of 4 mm. were prepared from African white diamonds by Drs. P. Grodzinski and J. H. F. Custers of the Research Department of Industrial Distributors, Ltd. The knives were mounted on a microtome especially built for the purpose, the most important feature of which is a thick steel bar mounted in accurately turned bearings. The specimen embedded in methyl methacrylate is held firmly in a small metal holder fixed eccentrically in a chuck at one end of the steel bar, which is rotated by a fractional horse-power electric motor. When the specimen is set in motion, a small electric heater in contact with the steel bar supplies sufficient heat to cause slight expansion of the steel, which slowly advances the specimen towards the knife. By revolving the specimen at controlled speeds the section thickness can be varied.

20. The Uptake and Retention of Fluoride by Enamel and Dentin. *S. A. Leach, M. R. C. Research Student, Turner Dental School, Manchester.* Powdered enamel and dentin take up fluoride rapidly from solutions of sodium fluoride (50 per cent of the uptake occurs in 15 minutes' shaking); this uptake is not greatly influenced by a concentration of sodium fluoride greater than 1 per cent. Simultaneously with the uptake of fluoride there is release of phosphate at a concentration less than the stoichiometric equivalent of fluoride adsorbed. In a saturated solution of sodium fluoride (4 per cent NaF) 1 per cent suspension of enamel and dentin take up 1.57 per cent and 1.80 per cent of the available fluoride, respectively. This is equivalent to a concentration of 2.8 per cent of fluoride in the enamel and 3.2 per cent of fluoride in the dentin. Over 50 per cent of the adsorbed fluoride is released from the enamel or dentin by shaking in successive washings of water, less fluoride being liberated at each successive washing until a level is reached where the fluoride is continually liberated at a concentration of 0.5 p.p.m. This concentration of 0.5 p.p.m. is not sufficient to affect the carbohydrate metabolism of *Staphylococcus aureus, Streptococcus viridans, Lactobacillus acidophilus,* and *Bacterium coli;* however, the fluoride remaining in the enamel and dentin can reduce their solubility in acid.

21. Skeletal Distribution of Fluorine. *S. M. Weidmann, Biological Research Unit, School of Dentistry, University of Leeds.* Young and adult rabbits were kept for various lengths of time on water containing comparatively high concentrations of fluorine. The uptake of fluorine was measured in the roots and crowns of molars and incisors, in cortical and cancellous parts of long bones, and in the alveolar bone. Differential uptake of fluorine was also estimated in bone-powder samples obtained from the endosteal and periosteal areas of the shaft diaphysis. The rate of fluorine uptake varies according to age, area, and type of the hard tissue.

22. Microradiographic Studies of the Organic Matrices of Dentine and Enamel. *R. C. Greulich, Institute for Physical Cell Research, Karolinska Institutet, Stockholm.* The amount of absorption from a polychromatic x-ray spectrum is a function of the dry mass of the absorber. This fundamental relationship has been applied to mass distribution studies of organic materials in histologic sections of decalcified teeth. A special x-ray apparatus has been constructed which includes a high-vacuum sample chamber and a 1,000 Å aluminum window. In the range of voltages applied routinely, approximately 30 per cent of the radiation is of 50 Å wave length. Formalin-fixed jaws from young rats, decalcified in EDTA (tetrasodium salt) buffered to pH 7.4, were sectioned in paraffin at 2 to 5 microns. Sections were mounted directly on the surface of Eastman Kodak Spectroscopic Plates (No. 649), and were then deparaffinized in chloroform. Exposure to x-irradiation followed at energies ranging from 1.2 to 1.8 kV, exposure time and energy applied being dependent upon the section thickness. The resultant natural-sized microradiograms possessed such high resolution that they could be examined in the light microscope at magnifications up to 1,000 diameters. Examination of microradiograms of incisors and molars has revealed several features of mass distribution which cannot be seen in histologic preparations. These include the presence of a membrane of relatively high mass approximately 1 micron in thickness lying between the ameloblasts and the completed enamel matrix; a number of dilatations along the length of dentinal tubules possessing very low mass; and an extremely low mass of organic materials in areas of pre-enamel and predentin.

23. The Relationship Between Chewing and the Secretory Activity of the Human Parotid Gland. *A. C. Kerr, Department of Dental Medicine, Guy's Hospital, University of London.* The reflex response of parotid glands to the chewing of paraffin wax was studied in 6 individuals. Secretion was collected by cannulae retained in the parotid ducts by suction. Secretion rates were measured by an integrating flow meter. When chewing was unilateral the output of the ipsilateral gland was markedly increased. The contralateral gland was only slightly stimulated. Because of this all stimulus response effects were studied unilaterally. Outputs at chewing rates between 40 and 90 strokes each minute were not appreciably different. Below 40 strokes per minute the secretion rate was lower. Above 90 strokes per minute

fatigue quickly developed and, although a slightly higher output was recorded for a minute or so, this could not be sustained. The chewing of 2g. pieces of wax of different melting points showed that the secretory response increased with the melting point. Wax melting at 56° C. provoked about 50 per cent more secretion than wax melting at 39° C. When pieces of wax varying in size from 0.02 to 2g. were chewed the secretory response was linearly related to the logarithm of the weight of the wax bolus. Chewing on imaginary pieces of wax produced no increase above the basal rates. The secretory response of the parotids to wax chewing thus depends upon the size of the piece of wax chewed, upon its plasticity, and upon the pattern of chewing with regard to the side of the mouth used. The output is not much affected by rates of chewing so long as they are within physiologic limits.

24. Studies on the pH of Plaque in Interproximal Areas After Eating Sweets and Starchy Foods. *G. N. Jenkins and I. Kleinberg, Department of Physiology, King's College, Newcastle-upon-Tyne.* The studies of Stephan (*J. A. D. A.*, **27**: 718, 1940; and elsewhere) on the fall of pH in the plaque on the surfaces of teeth after glucose rinses have been extended. A modified type of antimony electrode has been used which reaches well into interproximal areas, and is shaped so that the same area is reached each time it is placed in position. Stephan's results, with 10 per cent sugar solutions, were confirmed for interproximal areas. The pH of interproximal areas has been studied during and following the sucking of sweets and the eating of starchy foods under reasonably normal conditions. Measurements of salivary pH have also been made and the role of salivary pH in controlling that of the plaque has been assessed. The results, as a whole, tentatively suggest that during the consumption of these foods the pH drop is slow and the minimum pH is reached only after eating is finished. This delay in the pH drop is thought to be partly caused by the rise in pH of the saliva which occurs during the increased rate of flow resulting from the stimulus of eating. In addition, with sweets, the sugar in the mouth may reach sufficiently high concentrations to inhibit bacterial acid production. In subjects whose interproximal spaces retained bread after eating, the pH remained at a low level for a considerably longer time than in the classical Stephan curve. If the bread was not retained in a particular area, the pH drop was almost negligible.

25. The Isolation and Classification of Lactobacillus Species From Human Saliva. *G. H. G. Davis, A. C. Hayward, and K. A. Bisset, Department of Bacteriology, University of Birmingham.* The spectrum of Lactobacillus species found in 66 Italian saliva samples collected in Rome was similar to those reported for American, Scandinavian, and English salivas. Of 368 strains isolated, 357 were classified into the following species: *Lactobacillus casei, Lactobacillus plantarum, Lactobacillus salivarius* (Rogosa and associates, *J. Bact.* **65**: 681, 1953), *Lactobacillus acidophilus, Lactobacillus fermenti, Lactobacillus brevis,* and *Lactobacillus buchneri.* The classification system used was a simplified version of Davis' (*J. Gen. Microbiol.* **13**: 481, 1955). The most commonly

occurring Lactobacilli were of the *Lactobacillus casei, Lactobacillus acidophilus,* and *Lactobacillus fermenti* types. Strains of *Lactobacillus brevis* were of 2 distinct types, one being the common saprophytic *Lactobacillus brevis* of cheese, silage, etc., and the other, which occurred more regularly in the mouth, being apparently truly parasitic and thus resembling *Lactobacillus acidophilus.* Strains of all the other 5 species occur in nonoral sites. The incidence of *Lactobacillus acidophilus, Lactobacillus brevis,* and *Lactobacillus buchneri* was significantly greater in samples after 96-hour enrichment culture in T. J. broth than in the same samples after only 24 hours, emphasizing the importance of adequate technics in surveys of this type. Five samples yielded strains of Leuconostoc, an organism not previously recorded from saliva. Of 19 salivas from caries-free mouths, 7 were apparently devoid of Lactobacilli. Seven of the 47 carious salivas also yielded no Lactobacilli. The relatively high proportion of Lactobacillus negative samples in the caries-free group is an interesting feature of these results.

26. Technic for Studying the Changes in the Flora of Extracted Teeth When Irrigated Continuously with a Nonnutrient Medium. *R. L. Hartles and B. J. Wagg, Department of Dental Biochemistry, School of Dental Surgery, University of Liverpool.* An apparatus has been designed for studying the changes in the flora of an extracted tooth in vitro during continuous slow irrigation with Krebs-Ringer saline. The apparatus is constructed in glass and consists of a tube in which the tooth with the crown uppermost rests on a constriction. The irrigating fluid is contained in a side-arm reservoir and passes over the tooth via a plug of asbestos and a string wick. The fluid then collects in the base of the tube. The rate of flow varies between 2 to 5 ml. per 24 hours. A syphon tube sealed into the base provides a means of emptying the solution after irrigation without disturbing or contaminating the tooth. Experiments performed on a small series of extracted teeth irrigated with the saline for 6 to 8 weeks indicated that the majority of oral microorganisms rapidly die under these conditions. In general, no organisms could be cultivated from clinically sound teeth. Lactobacilli were frequently isolated from teeth with early carious lesions. A mixed flora of yeasts and Staphylococci was obtained from grossly carious teeth and from those with appreciable amounts of adherent periodontal debris. None of the teeth yielded Streptococci, even after only 3 to 5 days' irrigation. All the Lactobacilli isolated belonged to the casei-plantarum group. Observations suggested that in a few of the teeth there was some increase in the size of the carious lesions during the 6- to 8-week period of irrigation.

27. Boric Acid as a Histochemical Blocking Reagent. *P. H. Staple, M. R. C. External Staff, University College Hospital Medical School, Dental Department, London.* Histochemical reactions of polysaccharides (Staple, *Nature, Lond.* **176**: 1125, 1955) are being further studied to see whether the formation of boric acid complexes resistant to oxidation by lead tetracetate is influenced: (a) within individual sugar radicles by the nature of groups adjacent to the

reactive hydroxyls; and (b) by the arrangement of the reactive sugar radicles within the polysaccharide chain(s). Besides the galactogen and glycogen originally studied, ivory nut mannan and alginic acid have been incorporated in gelatin films. After oxidation, such preparations have been stained with benzidine or ethanolic Schiff's reagent. Boric acid blocks the oxidation of all the polysaccharides studied. Whereas this might be expected for those containing reactive adjacent *cis*-hydroxyls (Böeseken, *Adv. Carbohydrate Chem.* **4**: 189, 1949), the reaction of glycogen, apparently anomalous, may be similar to that reported by Foster, Newton-Hearn, and Stacey (*J. Chem. Soc.*, p. 30, 1956). Contrary to Glegg, Clermont, and Leblond (*Stain Technol.* **27**: 277, 1952), glycogen in liver sections and films, after treatment with lead tetracetate at room temperature, does stain with Schiff's reagent, provided the latter is made up in ethanolic solution. Sections of the albumin gland of Helix pomatia react similarly to films of purified galactogen extracted from albumin glands. These findings are relevant to the elucidation of the action of "pectinase" on gingival tissue.

PAPERS READ BY TITLE

28. Observations on the Inhibition of Salivary Glycolysis. *R. L. Hartles, Department of Dental Biochemistry, School of Dental Surgery, University of Liverpool.* Manometric methods were used to determine the effect of 4 inhibitors on salivary glycolysis. The substances were sodium ricinoleate (I), sodium lauryl sulphate (II), sodium N-lauryl sarcosinate (III), and sodium potassium copper chlorophyllin (IV). All were added in a concentration of 67 p.p.m. The effect on glycolysis was observed first, when the inhibitor was added to the saliva before the addition of glucose, and secondly, when added after the addition of the sugar. The percentage inhibitions were as follows: (I) 57, 11; (II) 75, 18; (III) 10, 0; (IV) 76, 77. With the exception of IV the inhibition of glycolysis was much less when the inhibitor was added after the glucose. In a sample of whole saliva, therefore, a given concentration of inhibitors I, II, or III is more effective when added before the substrate than when added to the actively glycolysing saliva. In IV the degrees of inhibition is unchanged.

29. The Carbohydrate Content of Human Dental Enamel. *M. V. Stack, M.R.C. (External) Staff, Dental School, University of Bristol.* Levels of carbohydrate in the outermost region of thoroughly cleaned enamel were found to be at least 10 times those of the main body of enamel. This layer was ground off in order to determine the range in carbohydrate content of representative enamel. Samples were prepared by flotation from 30 permanent and 20 permanent teeth, 15 ± 5 (S.D.) mg. per cent, was not significantly different carbohydrate, which reacted as glucose, were shown by analyses with solutions of orcinol or of anthrone in 70 per cent sulphuric acid. The mean value for permanent teeth, 15 ± 5 (S.D.) mg. per cent, was not significantly different from that for deciduous teeth (16 ± 5 mg. per cent). Enamel from permanent

molars was found to contain two-thirds as much carbohydrate as that from other types of permanent teeth. This observation was supported by tests on pairs of teeth from 5 patients, and was thought to depend on the smaller surface:volume ratio of molar teeth, and on the greater proportion of carbohydrate in the outer region of the enamel. Correlations were not observed between carbohydrate levels and age or caries experience. The carbohydrate content of the enamel from 5 pairs of teeth from 1 individual was 15 ± 2 mg. per cent. Samples of developing enamel in which the organic contents were 10, 20, and 40 times the level in mature enamel were found to contain 7, 10, and 13 times as much carbohydrate, respectively. Pooled "chalky" enamel, in which had been found 3 to 4 times as much organic matter as normal enamel (Stack, *Brit. D. J.* **96**: 73, 1954), showed a carbohydrate level 12 times greater than normal.

30. The Enzymatic Activity of Radiated Exteriorized Salivary Glands. *J. A. English, Naval Medical Research Institute, Bethesda 14, Maryland.* It was previously determined that when salivary glands of rats were radiated in situ with 3 to 5000 r. of 200 Kv. x-ray there was a significant increase in specific activity of the glucose-6-phosphate and isocitric dehydrogenases. At the same time it was observed that there were other more general effects from this localized radiation. The present study was designed to determine what effect radiation (2,000 r.) would have upon the enzyme systems of exteriorized salivary glands of rats under conditions wherein the rest of the animal was completely shielded. Under these conditions we observed no general effects upon the animals, and even the weights of the radiated salivary glands were not significantly altered, whereas in the previous study this was not the case. The values for glucose-6-phosphate dehydrogenase were higher for radiated salivary glands in 17 out of 18 groups of animals, the average values in arbitrary units being 3.36 ± 0.71 for the nonradiated glands and 3.83 ± 0.67 for the radiated glands when all groups of rats were considered (probability 0.05). During the second week following radiation, the increase in activity of this enzyme was greater, the values being 3.04 ± 0.48 and 3.80 ± 0.42, respectively (probability 0.01). There was not a significant change in isocitric dehydrogenase following radiation of the exteriorized salivary glands.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section

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Compiled by Frances Krasnow, *Editor, New York Section*

1. An Anthropologic Study of Dental Anomalies. *Meyer Klatsky, New York.* This study is based on the examination of 8,328 human skulls of various ethnic stocks of ancient, primitive, and modern peoples. Twenty-five geographically arranged groups were investigated in the Department of Anthropology of the American Museum of Natural History, New York. The incidence of 6 different dental anomalies was studied: torus palatinus, torus mandibularis, supernumerary teeth, genetically missing teeth, reduced teeth, and impacted teeth. The number, location, and character of each one of these anomalies was observed and recorded. Following are the results: Torus palatinus was found in 18 of the geographic groups examined, totalling 482 cases. A particularly large number of cases with this anomaly was found among the German, Austrian, and Hungarian groups, and also among several of the American Indian collections, and the Eskimos. Torus mandibularis was noted in 11 groups, totalling 89 cases. The occurrence of this anomaly seems to be limited to some American Indian tribes, Eskimos, and Aleutian Islanders. Supernumerary teeth were found in 14 groups, totalling 41 teeth. Genetically missing teeth were found in all the 25 groups, totalling 947 teeth. Reduced teeth were found in 20 groups, totalling 129 teeth. Impacted teeth were found in 19 groups, totalling 157 teeth.

2. Clinical Evaluation of High Speed Rotating Instruments. *Chester J. Henschel, Sydenham Hospital, New York.* Instrument surface speed of 5,000 feet per minute or more is required and is demonstrable in vitro for maximum cutting efficiency. To reach this, a ¾ inch disc must attain nearly 30,000 r.p.m. and a 1/16 inch diameter bur or stone about 300,000 r.p.m. Definition of high speed must be based on surface speed of each instrument; engine speed is irrelevant. At these speeds coolants and lubricants at site of cutting are mandatory. Vernier control of these, and improvement in bearings, power transmission, and motors is imperative. Increased care of all moving parts is necessary to avoid frequent breakdown. Total time required to cut tooth in vivo may be shortened, but time saved is percentage of about 10 per cent of operating day when dental engine runs. Digital pressure may be greatly reduced, tactile sense generally enhanced, amplitude of vibration lowered, fear and pain lessened, and smaller-diameter, delicate, and adaptable instruments made efficient. Because of unrefined available coolant apparatus, limitations of sleeve and ball-type bearings, danger from too rapid cutting, and loss of "feel" at fractional

ounce pressures, less than maximum surface speed may be desirable. Present technic accepts as little as 50 feet per minute using 1/16 inch stones, so any marked speed-up is dramatic improvement. Humane and practical considerations impel the use of improved, more rapid, but less than maximum cutting efficiency in vivo.

3. Proportionate Relevance of Factors in Human Caries. *H. H. Neumann and N. A. DiSalvo, School of Dental and Oral Surgery, Columbia University.* A study of caries prevalence in aboriginal tribes of the western hemisphere led to similar conclusions as previous observations in other areas; that the different known caries factors are greatly varying in the degree of their influence on caries prevalence. Assessing in addition to our findings also reports in the literature these factors may be grouped as follows: (1) marginal influences on caries prevalence (at a ratio of 1 to 1.5 or less), which factors include race, climate, nutritional balance, hormonal balance, oral hygiene; (2) the fluorine level in the drinking water, influencing caries prevalence at a rate of about 1 to 2; (3) the effect of "natural" versus "refined" diets, influencing the caries prevalence at a ratio of 1 to 100. We encountered tribes with an average DMF of 0.1 in adults, living on unbalanced diets, deficient in nutritional essentials, including calcium and proteins, high in carbohydrates, in some areas even simple sugars, with no knowledge of oral hygiene and on a water supply very low in fluorides. No racial immunity is at play as members of the same tribes are subject to rampant decay if they adopt a modern diet. The difference between "natural" and "refined" diets may be in the much emphasized destructive properties of the latter, in a protective agent common to natural diets, or likely in combination of both. The sole common denominator in the widely varying "natural" diets was a biophysical nature: the load applied on the teeth. Its suspected influence is not through a cleansing action but through a phenomenon well known in physics and biology; the effect of intermittent loads on structures and crystal orientation, viz., on posteruptive enamel maturation.

4. The Role of the Stratum Intermedium in Tooth Development. *Percy L. Johnson and Gerrit Bevelander, New York University College of Dentistry, and The Murry and Leonie Guggenheim Dental Institute.* The role of the stratum intermedium in tooth development has been somewhat obscure. In a study of pre-erupted and erupting embryonic pig teeth, it was found that the single-layered stratum intermedium undergoes rapid proliferation near the erupting cusp, forming a multicellular layer. This layer subsequently, during eruption, establishes linkage with the oral mucosa through its active growth across the intervening connective tissue. While this is occurring the degenerating ameloblasts and the oral epithelium, destined to elaborate the enamel cuticle, continue occlusally along the crown of the tooth. The stratum intermedium in its proliferation elaborates intercellular bridges fusing with the degenerating ameloblasts which likewise, in turn, develop intercellular bridges, all forming a complex, laying down the epithelial attachment of the tooth.

5. In Vitro Calcification of Aorta and Other Soft Tissues. *Bertram Eichel, Albert E. Sobel, Martin Burger, and Stanley W. Morgenstern, Jewish Hospital of Brooklyn, New York.* Several tissues containing both collagen and a mucopolysaccharide were incubated at 37° C. in inorganic solutions employed for calcification in vitro. As measured by silver stain, rat tail tendon, rat and rabbit aortae mineralized in vitro, whereas rat nasal septum cartilage did not. The study of the aorta was expanded. After 20 hours of calcification in vitro, the silver stained rat and rabbit aortae are characterized in the cross section by a light black ring in the media. In the rabbit, this stain extends into the intima. This silver staining is more intensive with longer periods in the calcifying solutions. Histopathologic examinations of the mineralized rat aorta resembles the medial calcification found in Mönckeberg's disease, without the accompanying necrosis. Chemical analyses showed a Ca/PO_4 ratio of about 2.0, the calcium being from 2 to 8 per cent of the dry weight. The Ca/PO_4 ratios resemble those approximately found in normal bone and teeth. These findings indicate the existence of a system present in the aorta that is capable of mineralization in vitro and may represent a clue to the mineralization of the aorta occasionally found in vivo.

6. The Effect of Bone Bank Preservation on the Calcifying Mechanism. *Martin Burger, Leroy S. Lavine, Albert Hirschman, and Albert E. Sobel, Jewish Hospital of Brooklyn and State University College of Medicine at New York City.* To test the influence of bone bank preservation methods on the calcifying mechanism, calcification in vitro of rachitic rat cartilage was employed. It was found that the present methods of bone storage inactivate the calcifying mechanism. This inactivation was reversible for some methods of bone preservation. The methods studied of preserving bone were: (1) deep freeze, (2) aqueous merthiolate, and (3) the boiled bone technic. All of these methods are utilized for storing bone for orthopedic use. When preserved bone was placed in a calcifying medium, new mineralization was zero compared to controls of fresh bone which showed excellent calcification. However, when preserved bone was treated with a calcium chloride solution, the calcifying mechanism was reactivated; most easily in deep freeze bone, next, in merthiolate treated bone, and least in boiled bone. This effect is further corroborated by studies of metachromasia. The calcifying mechanism survived in those bones first treated with calcium chloride solution and then frozen. The results of these studies indicate that the value of stored bone may be enhanced by proper chemical treatment; this fact has important clinical significance.

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I.A.D.R.—35th Annual Meeting
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March 21, 22, 23, 1957

Dental Materials Group
March 22, 23, 24, 1957
Registration Fee $3.00

Preprinted abstracts will be supplied before the meeting to those who register by mail prior to February 15, 1957. Write to

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INDEX

Volume, 35, 1956

ACID: amino; 255, 260, 263
——: salivary sediment, fluoride, 197
Acid formation: dental plaques, 778, 786, 792
——: ——; salivary sediment; sugar, 220
Aciduric bacteria: 404
——: environment; establishment, 596
Adamantinoma: mucoepidermoid variation, 955*
Adenitis: obstructive; experimental, 852
ADLER, P.: caries incidence; adolescents; occlusion, 344
Adrenal cortex: histochemistry, 547
ALLEN, T. J.: dental restorative materials; antibacterial effects, 25
ALTINSEL, H. H.: antibiotics; endodontics and operative procedures, 914
Aluminum oxide: effect on lungs, 693
Amalgam restorations: residual mercury; strength, 458
Ameloblastoma: (see adamantinoma)
Amino acid composition: dentin, 255
——: ——; variations, 263
——: enamel, 260
——: ——; variations, 263
Ammonia: saliva, 438
Amylase: saliva; determination, 391
ANDERS, J. T.: chloride level; saliva, 753
——: dimethylaminobenzaldehyde; salivary tryptophane measure, 241
——: salivary acidity; alkalinity; caries, 385
ANDERSON, D. J.: mastication stress, 671
——: stress measurement; mastication, 664
Anesthesia: inferior alveolar nerve block; epinephrine effects, 696
——: procaine, 696
——: Xylocaine, 824
Anomalies: anthropologic study, 968*
Antibiotics: endodontics, 656
Anti-enzyme action: fluoride, 189
Antiseptics: endodontics, 656
Aorta: calcification, 970*
Arch width: 4 to 8 years, 879
ARNIM, S. S.: pH; dental plaque; saliva, 83
ASGAR, K.: chemical analysis; teeth, 742

BACTERIA: aciduric, 404, 596
——: antibacterial effects; restorative materials, 25
——: antibiotic effect, 399
——: biochemical studies, 603
——: chondrosulfatase production, 299
——: "fusospirochetal" exudate; experimental injection, 4
——: pH levels; glucose solutions, 226
——: saliva; caries-susceptible; caries-immune, 572
——: sorbitol fermentation, 233
——: (see also specific names)
Bacterial flora: changes; extracted teeth, 965*
BAINS, M. E. D.: residual monomer, polyethyl methacrylate, 16
BARETTO, M. A.: Oral hygiene habits; periodontal disease, 645
BARTHA, E.: rat incisor; eruption; prolonged sleep effect, 562
BATTISTONE, G. C.: amino acid composition; dentin; enamel; variations, 263
——: ——; dentinal protein, 255
——: ——; enamel protein, 260
BENARDE, M. A.: rat saliva collection, 326
BEN SHUSHAN, D.: caries prevalence; Israel, 630
BEVELANDER, G.: pulpal calcification; histogenesis and histochemistry, 714
——: stratum intermedium; tooth development, 969*
BHASKAR, S. N.: experimental obstructive adenitis, 852
——: mucoceles; pathogenesis, 863
——: squamoso-mandibular articulation; development; rat, 533
Birth season: primary dentition, 909
BISSET, K. A.: salivary lactobacilli; classification, 964*
BIXLER, D.: adrenal cortex; histochemistry; salivary gland removal, 547
——: caries activity; desalivated rats, 615
BLACKWELL, R. Q.: sucrose effects; enamel solubility, 210
BLAKE, G. C.: diffusion pathways; cementum; dentin; enamel, 961*
Blood loss: oral surgery, 175
Blood vessels: pulp, 957*
BOLDEN, T. E.: experimental obstructive adenitis, 852
——: mucoceles; pathogenesis, 863
Bone: oxidase, 276
——: peroxidase, 276
——: phosphatase, 276
——: starch sponge; implant, 446
Bone bank: calcifying mechanism, 970*
Boric acid: histochemical blocking; reagent, 965*
BOWEN, R. L.: epoxy resins; restorative material, 360
BRAIN, E. B.: nerves; silver staining; urea solutions, 958*
BREWER, H. E.: enamel permeability; fluorides, 59
BRUDEVOLD, F.: fluoride distribution; enamel, 420
——: lead distribution; enamel, 430
——: resin composition; bonding to dentin, 846
——: tin distribution; enamel, 749
BUONOCORE, M.: resin composition; bonding to dentin, 846
Bur: inverted cone design factor effects, 704

*Abstract.

BURNETT, G. W.: amino acid composition; dentinal protein, 255
——: ——; dentin; enamel; variations, 263
——: ——; enamel protein, 260
——: dissolution rate; inorganic dentin; fluorides, 413
——: errata, 165
——: reducing activity; lactobacilli, 370
BURGER, M.: bone bank preservation; calcifying mechanism, 970*
——: in vitro calcification; aorta; soft tissues, 970*
Bushmen: primitive; acid formation; plaque, 786

CALCIFICATION: aorta, 970*
——: pulp; histogenesis and histochemistry, 714
——: sequence; mandibular molars and premolars, 555
——: soft tissues, 970*
Calcifying mechanism: bone bank preservation, 970*
Calcium: radioactive; tooth mineralization, 147
Carbohydrate: enamel, 966*
——: food; oral rinsing; anthrone method, 90
Caries: acid formation; immune individuals, 792
——: activity tests; evaluation, 332
——: arrested, 956*
——: desalivated rats, 615
——: diagnosis; radiographs, 33
——: diet, 39, 95, 638
——: distribution; rats; sexual similarity, 286
——: factors; proportionate relevance, 969*
——: fissure widths, 620
——: fluorides, 49, 54
——: food habits, 638
——: gingival plaque; hamster, 651
——: hamster, 95, 651
——: Israel, 630
——: lactic acid formation; plaques, 778
——: lactobacilli; Hunt-Hoppert rats, 586
——: nutrition, 39, 95, 638
——: occlusion; incidence, 334
——: punched-card records, 950
——: rat, 39, 286, 615, 586
——: saliva; acidity and alkalinity, 385
——: ——; immune and susceptible, 572
——: sodium fluoride, 54
——: sodium silico-fluoride, 54
——: stannous-fluoride dentifrice, 49
CARTER, W. J.: dental plaque; acid formation; caries-active individuals, 778
——: ——; ——; caries-immune individuals, 792
Cavity liners: plastic emulsions, 956*
Cavity walls: surface appearance; instruments, 509
Cementum: differential stain, 491
——: diffusion pathways, 961*
CHARLTON, J.: lactobacillus isolation; efficiency; two media, 800
Chewing: salivary secretory activity, 963*
Chloride level: saliva, 753
Chondrosulfatase production: oral bacteria, 299
CLARK, C. A.: aluminum oxide; effect on lungs, 693
Classification: teeth, 961*
CLAYCOMB, C. K.: laboratory tests for caries activity; evaluation, 332
——: salivary amylase; determination, 391
CLEGG, C. T.: ammonia production; saliva, 438
——: lactic acid production; saliva, 612
CLEMENTS, A. J.: dental plaque; acid formation; primitive Bushmen, 786
Composition: teeth, 742
——: ——; ammonia acid, 255, 260, 263
Condyle: mandibular; remolding, 137
Connecticut: caries; food habits, 638
COOKE, B. E. D.: oral epithelium; nevi, 954*
COSTICH, E. R.: inferior alveolar nerve block anesthesia, 696
Cotton fibers: operative wounds, 956*
COWAN, A.: anesthesia; Xylocaine evaluation, 824
COX, G. J.: punched-card records; carious lesions, 950
CRABB, H. S. M.: x-ray investigations; developing enamel, 960*
CRAWFORD, W. H.: fluid penetration; fillings and teeth, 518
CROWLEY, R. E.: personality factors; cooperation in dental treatment, 157
Cultivation: in vitro; tooth germs, 440, 523, 960*
CUNAT, J. J.: squamoso-mandibular articulation; development; rat, 533
Cuspal and palatal slopes of teeth: inclined planes of glenoid fossa, 890
Cynatosis: periodontal findings, 840

DALDERUP, L. M.: caries; nutrition; histologic investigations, 39
DALL, R.: caries; food habits; Connecticut, 638
DAVIES, G. M.: dental conditions; Polynesians; malocclusion, 115
——: periodontal disease; Polynesians, 734
DAVIS, G. H. G.: salivary lactobacilli; classification, 964*
DAY, H. G.: stannous-fluoride dentifrice; caries; adults, 49
DE MARCO, C.: fluoridation; enamel solubility, 185
Dental lamina: 959*
Dental plaque: acid formation, 786, 792
Dentin: amino acid composition, 255, 263
——: bonding of resins, 846
——: chemical analysis, 742
——: diffusion pathways, 961*
——: fluoride, 205, 413, 962*
——: lipid, 273
——: microradiographic studies, 963*
——: organic matters; ethylenediamine vs. KOH-glycol, 947
——: oxidase, 276
——: peroxidase, 276
——: phosphatase, 276
——: protein; amino acid composition, 255

Design factor: inverted cone bur, 704
DEUSCHLE, F. M.: congenital dentofacial malformations; maternal dietary deficiency, 674
Diagnosis: caries; radiographs, 33
Diamond knife: ultrathin sections, 962*
DIENSTEIN, B.: biometric analysis; root development and eruption, 479
Diet: caries, 39, 95, 638
——: fats; odontogenesis, 930
——: maternal deficiency; dentofacial malformations, 674
Diffusion pathways: enamel; dentin; cementum, 961*
Dimethylaminoaldehyde: salivary tryptophane; measure, 241
DI SALVO, N. A.: proportionate relevance; human caries factors, 969*
DIXON, A. D.: maxilla; early developments; enamel, 959*
DOCTOR, R. H.: oral hygiene habits; periodontal disease, 645
DODD, M. C.: bacterial flora; caries-susceptible and caries-immune saliva, 572
——: sorbitol fermentation; oral bacteria, 233
DREIZEN, S.: penicillin-sensitive oral bacteria; protection; yeasts, 249
DUNN, J. K.: dental plaque; acid formation; caries-active individuals, 778

EDITOR'S Viewpoint: 1, 167, 329, 495, 661, 821
EICHEL, B.: in vitro calcification; aorta; soft tissues, 970*
Electrometric studies: gingiva; pregnancy, 566
Enamel: abnormal; formation; vaccinia, 685
——: amino acid composition, 260, 263
——: carbohydrate content, 966*
——: defects; birth season, 909
——: diffusion pathways, 961*
——: fluoride; anti-enzyme action, 189
——: ——; distribution, 420
——: ——; organic and inorganic fractions, 205
——: ——; uptake, 962*
——: Hunger-Schreger bands, 804
——: lead distribution, 430
——: lipid, 273
——: microradiographic studies, 963*
——: permeability; fluorides, 59
——: protein; amino acid, 260
——: solubility; fluoridation, 185
——: ——; sucrose effect, 210
——: tin, 749
——: x-ray investigations, 960*
Enamel cuticle: 313
Enamel organs: cells, 960*
Endodontics: antibiotics, 659, 914
——: antiseptics, 656
ENGEL, M. B.: gingiva; pregnancy; electrometric studies, 566
ENGLANDER, H. R.: dental plaque; acid formation; caries-immune individuals, 792
ENGLISH, J. A.: enzymatic activity; radiated salivary glands, 967*
Enzymatic activity: salivary glands, 967*
Epinephrine: effect in inferior alveolar nerve block anesthesia, 696
Epithelial attachment: study method, 308
Epoxy resins: 360
ERLENBACH, F. M.: caries; food habits; Connecticut, 638
Eruption: biometric analysis; rat molar, 479
——: rat incisor; prolonged sleep effect, 562
ESSELBAUGH, N. C.: caries experience; Washington State College; students, 132
Estrogen: urinary; periodontosis, 648
Ethylenediamine: organic dentin, 947
ETTLEMAN, L.: ethylenediamine vs. KOH-glycol; organic dentin, 947

FABER, J. E., JR.: aciduric oral micrococci, 404
FABIAN, F. W.: rat saliva collection, 326
FAIRHURST, C. W.: hygroscopic expansion, 499
FAJARDA, O. P.: endodontics; antiseptics; antibiotics, 656
FARMER, E. D.: recurrent ulcerations of oral mucosa, 954*
Fats: odontogenesis, 930
FEARNHEAD, R. W.: ultrathin sections; diamond knife, 962*
FINN, S. B.: fluoridation; enamel solubility, 185
FISCHER, R. B.: enamel permeability; fluorides, 59
——: x-ray fluorapatite pattern; fluoride treatment, 773
Fissure widths: caries, 620
FITZGERALD, R. J.: aciduric oral micrococci, 404
FLANAGAN, F.: mandibular condyle remolding; osteoclasts, 137
Fluorapatite: pattern; x-ray, 773
Fluoride: acid formation; salivary sediment, 197
——: anti-enzyme action, 189
——: dentin uptake, 962*
——: enamel distribution, 420
——: enamel permeability, 59
——: enamel solubility, 185
——: enamel uptake, 962*
——: fluorapatite pattern, 773
——: inorganic dentin dissolution, 413
——: lactobacilli, 939
——: organic and inorganic fractions; dentin and enamel, 205
——: pulp effects, 69
——: salivary gland effects, 922
——: skeletal storage, 65
——: sodium; caries, 54
——: ——; protozoa rotifera, 763
——: sodium silico; caries, 54
——: stannous; dentifrice; caries, 49
——: ——; urinary fluoride levels, 760
FOSDICK, L. S.: dental plaque; acid formation; caries-active individuals, 778
——: ——; ——; caries-immune individuals, 792
——: ——; ——; primitive Bushmen, 786
——: sucrose effects; enamel solubility, 210
FRIEDLAND, R.: periodontosis; urinary estrogen, 648

"Fusospirochetal" material: experimental injection, 4

GAIRNS, F. W.: sensory nerve endings; gingiva; palate; tongue, 958*
GALAGAN, D. J.: caries diagnosis; radiographs, 33
GANS, B. J.: gingiva; pregnancy; electrometric studies, 566
GARDNER, D. E.: fluoride distribution; enamel, 420
GARDNER, M. K.: laboratory tests for caries activity; evaluation, 332
GARN, S. M.: calcification sequence; mandibular molars and premolars, 555
GILMORE, R. W.: saliva; flow rate during sleep, 190
Gingiva: nerve endings, 958*
——: pregnancy, 566
Gingival crevices: bacteria; chondrosulfatase, 299
Gingival tissue: rough surface effect, 323
Gingivitis: "fusospirochetal" material, 4
——: rough surfaces, 323
Glenoid fossa: inclined planes; correlation with tooth slopes, 890
Glucose solutions: oral bacteria pH, 226
Glucose: (see also sucrose and sugar)
Glycolysis: salivary; inhibition, 966*
GOLDBERG, H. J. V.: carbohydrates; oral rinsing; anthrone method, 90
GOOSE, D. H.: maxillary permanent incisors; variability, 902
GORE, T. J.: saliva; physical and chemical properties, 102
GREEN, G. E.: bacterial flora; caries-susceptible; caries-immune saliva, 572
GREULICH, R. C.: microradiographic studies; enamel; dentin, 963*
GROSSMAN, L. I.: endodontics; antiseptics and antibiotics, 656

HANAHAN, L.: saliva; flow rate during sleep, 109
HANCOCK, J. A., JR.: abnormal enamel formation; vaccinia, 685
——: lymphocytic choriomeningitis; pregnancy; dental tissues; mice, 467
HARD, M. M.: caries experience; Washington State College; students, 132
HARGREAVES, G. L.: salivary sediment technic; caries inhibited; precision, 591
HARTLES, R. L.: flora changes; irrigated extracted teeth, 965*
——: salivary glycolysis; inhibition, 966*
HAYWARD, A. C.: salivary lactobacilli; classification, 964
HENRY, E. E.: design factors influencing performance of inverted cone bur, 704
HENSCHEL, C. J.: high speed rotating instruments; clinical evaluation, 968*
Heredity: sheep occlusion, 956*
HESS, W. C.: fluoride; organic and inorganic fractions; dentin and enamel, 205
——: lipid content; enamel; dentin, 273
——: phosphatase; peroxidase; oxidase; activity dentin; bone, 276
High speed rotating instruments: clinical evaluation, 968*
HIRSCHMAN, A.: bone bank preservation; calcifying mechanism, 970*
HITCHIN, A. D.: occlusion in sheep, 956*
HODSON, J. J.: adamantinoma; mucoepidermoid variation, 955*
HOLLOWAY, P. J.: tooth germ growth; vitamin A effects; tissue culture, 960*
HOPP, W. M.: dental arch width; 4 to 8 years, 879
HOPPERT, C. A.: fissure widths; caries; Hunt-Hoppert rats, 620
——: lactobacilli; comparative study; Hunt-Hoppert rats, 291
——: ——; frequency; Hunt-Hoppert rats, 586
——: penicillin and Terramycin; caries; bacteria, 399
——: rat saliva collections, 326
HUGHES, S. G.: tooth calcification, 961*
HUNT, H. R.: fissure widths; caries; Hunt-Hoppert rats, 620
——: lactobacilli; comparative study; Hunt-Hoppert rats, 291
——: ——; frequency; Hunt-Hoppert rats, 586
——: penicillin and Terramycin; caries; bacteria, 399
——: rat saliva collection, 326
HUNTER, H. A.: "fusospirochetal" material; experimental injection, 4
Hunter-Schreger bands: 804
Hydroxyapatite: saliva oversaturation, 245
Hygiene: oral; periodontal disease, 645
Hygroscopic expansion: 499

I.A.D.R.: annual meeting; executive proceedings, 814
——: meeting; New York section; proceedings, 968*
——: ——; British Division; participants, 954
——: ——; —— ——; proceedings, 1956, 954
——: new members elected, 818
——: officers elected, 818
——: supporting associates, 166, 328, 494, 660, 820, 971
Incisors: maxillary permanent; variability, 902
Inhibitors: acid formation; salivary sediment, 591
Instruments: cavity wall appearance, 509
IRVING, J. T.: dietary fats; tooth development, 930
Israel: caries, 630

JACKSON, D.: arrested dental caries, 956*
JACOBSON, F. L.: caries experience; Washington College students, 132
JENKINS, G. N.: plaque pH; sweets and starchy foods, 964*
JENSS, R. M.: blood loss; oral surgery, 175
JOHNSON, M.: gingival plaque; hamster, 651
JOHNSON, P. L.: stratum intermedium; tooth development, 969*
——: pulpal calcification; histogenesis and histochemistry, 714

JOHNSON, R. L.: blood loss; oral surgery, 175
JORDAN, H. V.: aciduric oral micrococci, 404
JORDAN, W. A.: caries inhibition; sodium silico-fluoride; sodium fluoride, 54
JOSEPH, N. R.: gingiva; pregnancy; electrometric studies, 566
J. D. Res.: supporting associates, 166, 328, 494, 660, 820, 971

KARLSON, K. E.: biochemical studies; oral bacteria, 603
KARSHAN, M.: periodontosis; ovarian function, 875
——: ——; urinary estrogen, 648
KERR, A. C.: chewing; salivary secretory activity, 963*
KEYES, P. H.: caries activity; whole-wheat ash diets; hamster, 95
KIFER, P. E.: fissure widths; caries; Hunt-Hoppert rats, 620
KLATSKY, M.: anthropologic study; dental anomalies, 968*
KLEBANOFF, S. G.: personality factors; cooperation in dental treatment, 157
KLEINBERG, I.: plaque pH; sweets and starch foods, 964*
KOH-glycol: organic dentin, 947
KONDO, W.: oral bacteria environment establishment; aciduric bacteria, 596
KORCHIN, L.: starch sponge; implant effects; bone, 446
KOYOUMDJISKY, E.: inclined planes of the glenoid fossa; cuspal and palatal slopes of teeth, 890
KRAMER, I. R. H.: I.A.D.R., proceedings; British Section, 954
——: plastic emulsions; cavity liners, 956*
——: pulp; vascular architecture, 957*
KRASNOW, F.: I.A.D.R.; proceedings New York section, 968
KRESHOVER, S. J.: abnormal enamel formations; vaccinia, 685
——: lymphocytic choriomeningitis; caries; pregnancy; dental tissue; mice, 467
KUMAMOTO, Y.: calcium; radioactive; tooth mineralization, 147
KYDD, W. L.: tongue forces; quantitative analysis, 171

LACTIC acid: saliva, 612
Lactobacilli: Hunt-Hoppert rats; comparative study, 291
——: ——; frequency, 586
——: isolation, 800
——: reducing activity, 370
——: saliva; classification, 964*
——: sodium fluoride effects, 939
LARSON, J. H.: fluid penetration; fillings and teeth, 518
LEACH, S. A.: fluoride uptake; enamel; dentin, 962*
Lead: enamel distribution, 430
LEBLOND, C. P.: calcium; radioactive; tooth mineralization, 147
LEE, C. Y.: lipid content; enamel; dentin, 273
LEFKOWITZ, W.: in vitro cultivation; rat tooth germ, 523
——: tooth germ cultivation; embryo extract-free medium, 440
LEOPOLD, R. S.: fluoride; organic and inorganic fractions; dentin and enamel, 205
LEVINE, L. S.: bone bank preservation; calcifying mechanism, 970*
LEVY, R. J.: differential stain; cementum, 491
LEWIS, A. B.: calcification sequence; mandibular molars and premolars, 555
LILIENTHAL, B.: anti-enzyme action; fluoride; enamel surface, 189
——: fluoride; effect on acid formation; by salivary sediment, 197
Lipid: dentin, 273
——: enamel, 273
LOBENE, R. R.: errata, 165
LOSEE, F. L.: ethylenediamine vs. KOH-glycol; organic dentin, 947
LUDWIG, T. G.: carbohydrates; food; oral rinsing; anthrone method, 90
Lungs: effects of aluminum oxide, 693
Lymphocytic choriomeningitis: pregnancy; dental tissues, 467

MACDONALD, J. B.: "fusospirochetal" material; experimental injection, 4
MAHLER, I. R.: pH levels; oral bacteria; glucose solutions, 226
Malformations: dentofacial; maternal dietary deficiency, 674
Malocclusion: Polynesians, 115
MANLY, R. S.: pH oral bacteria; glucose solutions, 226
——: salivary sediment technic; inhibitors; precision, 591
MANTZ, R. F.: dental restorative materials; antibacterial effects, 25
MARTIN, J. J.: biochemical studies; oral bacteria, 603
MARTIN, M. D.: anti-enzyme action; fluoride; enamel surface, 189
Mastication: stress, 664, 671
Materials: denture; residual monomer, 16
——: hygroscopic expansion, 499
——: restorative; antibacterial effects, 25
——: ——; epoxy resins, 360
MAURICE, C. G.: sodium fluoride; pulp effects, 69
Maxilla: early development; enamel, 959*
McCHESNEY, M. N.: salivary amylase; determination, 391
McDANIEL, W. L.: nerve distribution; maxillary teeth; variations, 916
McLEAN, J. W.: plastic emulsions; cavity liners, 956*
McSHANE, J.: endodontics; antiseptics and antibiotics, 656
MEHTA, R. S.: oral hygiene habits; periodontal disease, 645
MELLANBY, M.: tooth germ growth; vitamin A effects; tissue culture, 960*
Mercury: residual; amalgam restorations, 458
MEREDITH, H. V.: dental arch width; 4 to 8 years, 879

Methacrylate: polymethyl; residual monomer, 16
Microradiographic studies: dentin; enamel, 963*
Mineralization: tooth; radioactive calcium, 147
MITCHELL, D. F.: gingival plaque; hamster, 651
Monomer: residual; polymethyl methacrylate, 16
MOORE, B. W.: dental plaque; acid formation; caries-active individuals, 778
MORGENSTERN, W.: in vitro calcification; aorta; soft tissues, 970*
MORSE, E. H.: caries; food habits; Connecticut, 638
MORTELL, J. F., JR.: cavity walls; surfaces; instrument effects, 509
——: Hunter-Schreger bands, 804
MOSNY, J. J.: penicillin-sensitive; oral bacteria; protection; yeasts, 249
Mucoceles: pathogenesis, 863
MUHLER, J. C.: adrenal cortex histochemistry; following salivary gland removal, 547
——: caries activity; desalivated rats, 615
——: desiccated thyroid; propylthiouracil; testosterone; fluorine; submaxillary gland; rat, 922
——: enamel permeability; fluorides, 59
——: stannous-fluoride dentifrice; caries; adults, 49
——: urinary fluorine levels; stannous fluoride-containing dentifrice, 760
——: x-ray fluorapatite formation; fluoride treatment, 773
MYERS, H. I.: mandibular condyle remolding; osteoclasts, 137

NAPOLI, P. J.: personality factors; cooperation in dental treatment, 157
NATHANS, J. W.: punched-card records; carious lesions, 950
NEBERGALL, W. H.: stannous-fluoride dentifrice; caries; adults, 49
NEDERVEEN-FENENGA, M.: caries; nutrition; histologic investigation, 39
Nerve: distribution; maxillary teeth; variation, 916
——: endings; gingiva; palate; tongue, 958*
——: silver staining; urea solutions, 958*
——: supply; maxillary teeth, 958*
NEUMANN, H. H.: proportionate relevance; human caries factors, 969*
Nevi: oral epithelial, 954*
NIKIFORUK, G.: saliva; reducing properties; indicator dyes, 377
NOBLE, H. W.: cotton fibers; operative wounds, 956*
NOLTE, W. A.: pH; dental plaque; saliva, 83
Nutrition: caries, 39, 95, 638

OCCLUSION: caries incidence, 344
——: sheep, 956*
OCKERSE, T.: experimental periodontal lesions; white-tailed rat, 9
Odontogenesis: abnormal; vaccinia, 685
——: biometric analysis, 479
——: calcification; mandibular teeth, 555
——: dietary fats, 930
——: in vitro, 440, 523, 960*
——: lymphocytic choriomeningitis effect, 467
——: rat tooth, in vitro, 440, 523, 960*
——: stratum intermedium, 969*
——: vitamin A effect, 960*
——: x-ray investigations, 960*
ONISI, M.: oral bacteria environment establishment; aciduric bacteria, 596
Oral mucosa: recurrent ulcerations, 954*
Oral surgery: blood loss, 175
ORSÓS, S.: rat incisor; eruption; prolonged sleep effect, 562
Osteoclasts: 137
——: mandibular condyle remolding, 137
Ovarian function: periodontosis, 875
Oxidase: bone, 276
——: dentin, 276

PADDOR, O. R.: penicillin-sensitive; oral bacteria; protection; yeasts, 249
Palate: nerve endings, 958*
PECKHAM, S. C.: ethylenediamine vs. KOH-glycol; organic dentin, 947
——: lipid content; enamel; dentin, 273
——: fluoride; organic and inorganic fractions; dentin and enamel, 205
Penicillin: caries; bacteria, 399
Penicillin-sensitivity; oral bacteria; yeast protection, 249
Periodontal disease: classification and scoring, 350
——: oral hygiene habits, 645
——: Polynesians, 734
Periodontal lesions: experimental; white-tailed rat, 9
Periodontosis: cyanotic individuals, 840
——: "fusospirochetal" material, 4
——: ovarian function, 875
——: urinary estrogen, 648
Peroxidase: dentin; bone, 276
Personality factors: cooperation in dental treatment, 157
PETERSON, J.: caries inhibition; sodium silico-fluoride; sodium fluoride, 54
PEYTON, F. A.: cavity walls; surfaces; instrument effects, 509
——: Hunter-Schreger bands, 804
pH: oral bacteria; glucose solutions, 226
——: plaque; saliva, 83
——: ——; sweets and starch foods, 964*
——: (see also acid)
PHILLIPS, R. W.: residual mercury; amalgam restorations; strength, 458
Phosphatase: bone; dentin, 276
PIGMAN, W.: saliva; flow rate during sleep, 109
Plaque: dentobacterial; acid formation; sugar, 220
——: ——; lactic acid formation; caries-active individuals, 778
——: ——; pH, 83
——: gingival; hamster, 651
Plastic emulsions: cavity liners, 956*

PLOTKIN, R.: dental plaque; acid formation; primitive Bushmen, 786
Polynesians: dental conditions; malocclusion, 115
——: periodontal disease, 734
PORTER, D. R.: laboratory tests for caries activity; evaluation, 332
POTGIETER, M.: caries; food habits; Connecticut, 638
Pregnancy: gingiva, 566
——: lymphocytic choriomeningitis; mice, 467
Propylthiouracil: salivary gland effects, 922
Protein: dentin and enamel, 260
Protozoa: sodium fluoride effects, 763
Pulp: calcification; histochemistry and histogenesis, 714
——: nerves; accommodation, 723
——: reaction; electric current, 723
——: sodium fluoride effects, 69
——: vascular architecture, 957*
Punched-card records: carious lesions, 950

RADIATION: salivary glands; enzymatic activity, 966*
——: (see also radioactive, x-ray)
RADIKE, A. W.: stannous-fluoride dentifrice; caries; adults, 49
Radioactive calcium: 147
——: fluid penetration; fillings, 518
Radiographs: caries diagnosis, 33
RAE, J. J.: ammonia production; saliva, 438
——: saliva; lactic acid production, 612
RAGHEB, H. S.: lactobacilli; comparative study; Hunt-Hoppert rats, 291
——: penicillin and Terramycin; caries; bacteria, 399
RAMNARINE, K. C.: antibiotics; operative procedures and endodontics, 914
RANDLES, C. R.: sorbitol fermentation; oral bacteria, 233
RATHJE, W.: saliva; oversaturation; hydroxyapatite, 245
Reducing activity: lactobacilli, 370
Reducing properties: indicator dyes, 377
REEVE, W. L.: mandibular condyle remolding; osteoclasts, 137
Resin: bonding to dentin, 846
——: epoxy, 360
Restorations: cavity wall appearance, 509
——: fluid penetration, 518
RING, C. E.: x-ray fluorapatite pattern; fluorides, 773
ROBINSON, H. B. G.: Editor's Viewpoint, 1, 167, 329, 495, 661, 821
Root growth: biometric analysis; rat, 479
ROSEN, S.: lactobacilli frequency; Hunt-Hoppert rats, 586
——: lactobacilli; comparative study; Hunt-Hoppert rats, 291
——: penicillin and Terramycin; caries; bacteria, 399
Rotifera: sodium fluoride effects, 763
ROWLES, S. L.: nerves; silver staining; urea solutions, 958*
RUSSELL, A. L.: periodontal disease; classification and scoring, 350
RUSSELL, L. H.: pulp; vascular architecture, 957*
RYGE, G.: hygroscopic expansion, 499

SAINSBURY, H.: season of birth; primary dentition, 909
Saliva: acidity; caries, 385
——: alkalinity; caries, 385
——: ammonia, 438
——: amylase, 391
——: caries-susceptible; caries-immune; bacteria, 572
——: chemical properties, 102
——: chloride level, 753
——: desalivated rats; caries, 615
——: flow rate during sleep, 109
——: glycolysis; inhibition, 966*
——: lactic acid production, 612
——: lactobacilli, 572, 964*
——: over-saturation; hydroxyapatite, 245
——: pH, 83
——: physical properties, 102
——: rat; collection, 326
——: reducing properties; indicator dyes, 377
——: secretory activity; chewing, 963*
——: sediment; acid formation; fluoride, 197
——: ——; —— ——; sugar, 220
——: ——; technic, 591
——: tryptophane, 241
Salivary gland: desiccated thyroid; propylthiouracil; testosterone; fluorine effects, 922
——: effect on adrenal cortex, 547
——: obstructive adenitis, 852
——: mucoceles, 863
——: radiated; enzymatic activity, 967*
SALLAY, C.: periodontal findings; cyanotic individuals, 840
SANJANA, M. K.: oral hygiene habits; periodontal disease, 645
SCHATZ, A.: biochemical studies; oral bacteria, 603
SCHERP, H. W.: chondrosulfatase; production; oral bacteria, 299
SCHMIDT, E. G.: reducing activity; lactobacilli, 370
SCHNEYER, L. H.: saliva; flow rate during sleep, 109
SCHOCKLEY, T. E.: sorbitol fermentation; oral bacteria, 233
SCHOUR, I.: sodium fluoride; pulp effects, 69
SCHULTZ-HAUDT, S. D.: chondrosulfatase; production; oral bacteria, 299
SCHWEINSBERGER, R. A.: urinary fluorine levels; stannous fluoride-containing dentifrice, 760
SCOTT, J. H.: nerve supply; maxillary teeth, 958*
Sex: caries distribution, 286
SHAFER, W. G.: desiccated thyroid; propylthiouracil; testosterone; fluorine; submaxillary gland; rat, 922
SHAW, J. H.: caries distribution; sexual similarity; bilaterality; rats, 286
SHAY, D. E.: dental restorative materials; antibacterial effects, 25

SHIOTA, T.: lactobacilli; sodium fluoride effects, 939
SHOEMAKER, D. W.: calcification sequence; mandibular molars and premolars, 555
SINGER, J. L.: personality factors; cooperation in dental treatment, 157
Sleep: eruption rate, 562
——: salivary flow rate, 109
SMITH, D. C.: residual monomer; polymethyl methacrylate, 16
SMITH, F. A.: fluoride distribution; enamel, 420
SNYDER, M. L.: laboratory tests for caries activity; evaluation, 332
——: salivary amylase; determination, 391
SOBEL, A. E.: bone bank preservation; calcifying mechanism, 970*
——: in vitro calcification; aorta; soft tissues, 970*
Sorbitol: oral bacteria, 233
SPIES, H. C.: lactobacillus isolation; efficiency, 800
SPIES, T. D.: penicillin-sensitive; oral bacteria; protection; yeasts, 249
Squamoso-mandibular articulation: development; rat, 533
STACK, M. V.: carbohydrate content; enamel, 966*
Stannous fluoride: dentifrice, 49, 760
STAPLE, P. H.: boric acid; histochemical blocking; reagent, 965*
Starch sponge: implant; bone, 446
STEADMAN, L. T.: enamel; lead distribution, 430
——: enamel; tin distribution, 749
Stratum intermedium: tooth development, 969*
Stress: mastication, 664, 671
Sucrose: enamel solubility effects, 210
Sugar: acid formation; plaque; salivary sediment, 220
——: plaque pH, 964*
——: (see also glucose sorbitol and sucrose)
SUHER, T.: laboratory tests for caries activity; evaluation, 332
Supporting associates: I.A.D.R.; J.D.R., 166, 328, 494, 660, 821, 971
SWARTZ, M. L.: residual mercury; amalgam restorations; strength, 458
SWAYNE, P.: in vitro cultivation; rat tooth germ, 523
——: tooth germ cultivation; embryo extract-free medium, 440
SWEENEY, E. A.: caries distribution; sexual similarity; bilateralcy; rats, 286

TEETH: enamel; chemical analysis, 742
Temporomandibular joint: squamoso-mandibular articulation, 533
TEN CATE, A. R.: enamel organ cells, 960*
TENENBAUM, B.: periodontosis; ovarian function, 875
——: ——; urinary estrogen, 648
Terramycin: caries; bacteria, 399
Testosterone: salivary gland effects, 922
Thyroid: salivary gland effects, 922
Tin: enamel, 749
TONGE, C. H.: dental lamina, 959*
Tongue: forces; quantitative analysis, 171
——: sensory nerve endings, 958*
Tooth germs: cultivation in vitro, 440, 523, 960*
Tryptophane: saliva, 241
TULLAR, P. E.: acid formation from sugar; plaque; salivary sediment, 220
TURNER, N. C.: dimethylaminobenzaldehyde; salivary tryptophane measure, 241
——: saliva; acidity; alkalinity; caries, 385

ULCERATIONS: recurrent; oral mucosa, 954*
Urinary fluorine levels: stannous fluoride-containing dentifrice, 760

VACCINIA: abnormal enamel formation, 685
VARGAS, F. F.: normal and pathologic pulp nerves; accommodation, 723
VERMILLION, J.: caries diagnosis; radiographs, 33
Vitamin A: tooth germ effect, 960*

WAERHAUG, J.: enamel cuticle, 313
——: rough surfaces; gingival tissue effect, 323
WAGG, B. J.: flora changes; irrigated extracted teeth, 965*
WANTLAND, W. W.: parasites; protozoa; rotifera; sodium fluoride effects, 763
WARKANY, J.: congenital dentofacial malformations; maternal dietary deficiency, 674
WEBSTER, R. C.: adrenal cortex histochemistry following salivary gland removal, 547
WEDDLE, D. A.: fluorides; skeletal storage, 65
WEIDMANN, S. M.: fluorine; skeletal distribution, 963*
WEINMANN, J. P.: experimental obstructive adenitis, 862
——: mucoceles; pathogenesis, 863
——: squamoso-mandibular articulation; development; rat, 533
WEINREB, M. M.: caries prevalence; Israel, 630
WHITE, A. A.: phosphatase, peroxidase, oxidase activity; dentin; bone, 276
WIENER, R.: periodontosis; ovarian function, 875
WILEMAN, W.: resin composition; bonding to dentin, 846
WITKOP, C. J.: fissure widths; caries; Hunt-Hoppert rats, 620
Wounds: operative; cotton fibers, 956*

X-RAY: fluorapatite pattern; fluoride treatment, 773
X-ray investigations: developing enamel, 960*
Xylocaine: anesthesia, 824

YEASTS: protection; penicillin-sensitive; oral bacteria, 249

ZANDER, H. A.: epithelial attachment; study method, 308

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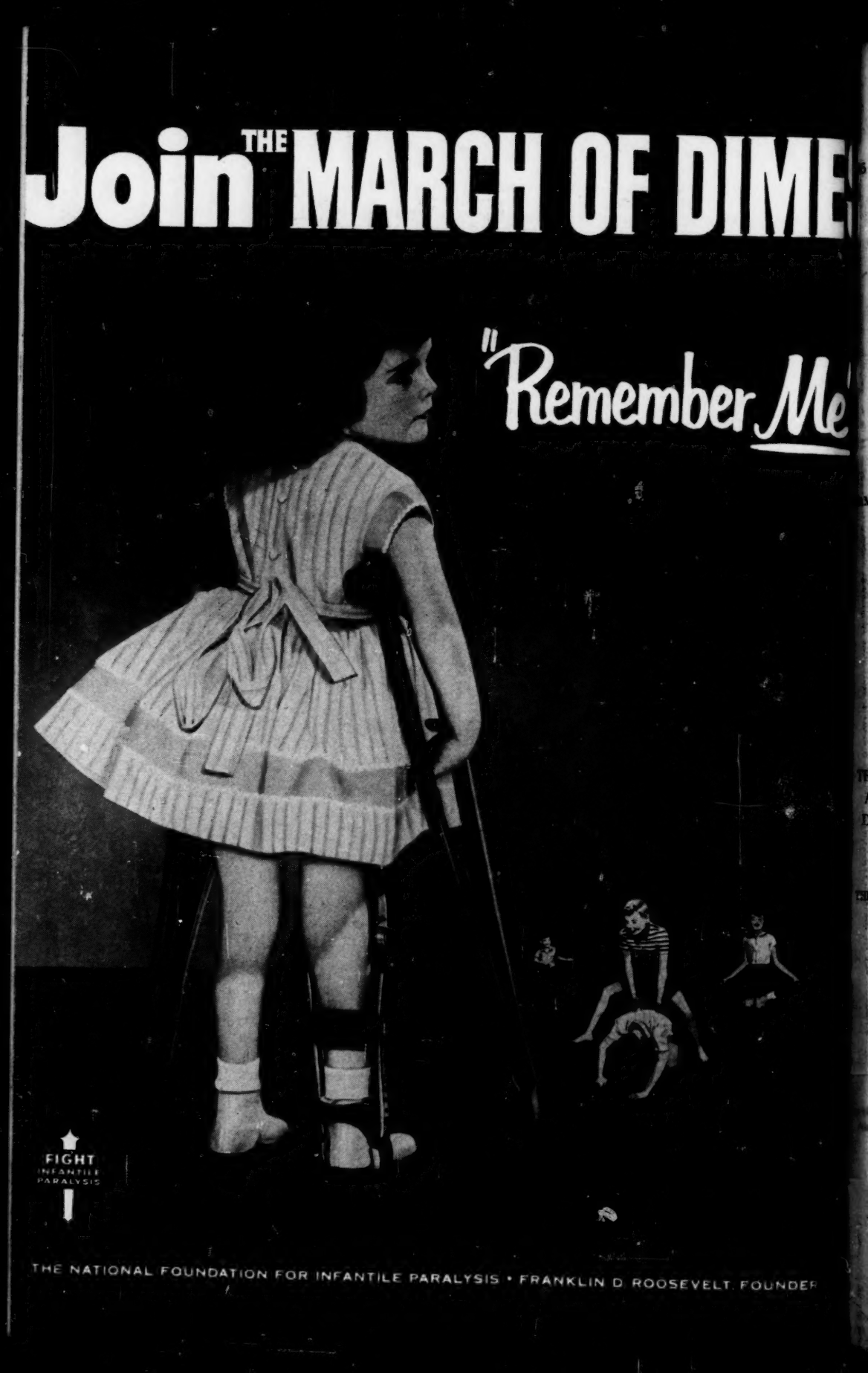

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MILTON B. G. ROBINSON

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Editor's Viewpoint ---------- 821

A Clinical Evaluation of Xylocaine Anesthesia. Adrian Cowan, M.B., B.Ch., B.Dent.Sc. (U. Dubl.), F.D.S.R.C.S. (England) (Royal College of Surgeons, Dublin, Ireland) ---------- 824

Periodontal Findings in Cyanotic Individuals. C. Sallay, M.D. (Stomatological Clinic of the University Medical School, Budapest, Hungary) ---------- 840

A Report on a Resin Composition Capable of Bonding to Human Dentin Surfaces. M. Buonocore, M.S., D.M.D., William Wileman, M.S., D.M.D., and Finn Brudevold, M.S., D.D.S. (Eastman Dental Dispensary, Rochester, N. Y.) ---------- 846

Experimental Obstructive Adenitis in the Mouse. S. N. Bhaskar, D.D.S., Ph.D., T. E. Bolden, D.D.S., M.S., and J. P. Weinmann, M.D. (University of Illinois College of Dentistry, Chicago, Ill.) ---------- 852

Pathogenesis of Mucoceles. S. N. Bhaskar, D.D.S., Ph.D., T. E. Bolden, D.D.S., M.S., and J. P. Weinmann, M.D. (University of Illinois College of Dentistry, Chicago, Ill.) ---------- 863

(Contents continued on inside back cover)

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